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VOLUME 23 NUMBER 2

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THE CAUSE OF THE REACTIONS FOLLOWING TRANSFUSION OF CITRATED BLOOD *

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AND
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BOSTON

In analyzing the reactions following blood transfusion by any of the different methods now in vogue, all figures should be based on cases in which donor and recipient have been properly matched prior to the operation. By means of the methods for grouping and direct matching given us by Moss[1] and others it is possible to exclude a certain number of gross hemolytic reactions which used to be inevitable. It is also a very simple matter to list a number of Group 4 donors, and with this done no emergency transfusion need ever become a completely reckless venture. In our experience it would appear possible to use carefully classified Group 4 donors for all cases, relying on the fact that the high dilution to which the incompatible plasma is brought nullifies its toxic effect. While this statement is in agreement with the views of Lee,[2] it is interesting to find it contradicted in the recent report of the Interallied Surgical Conference,[3] where it is stated that, "fatal accidents have occurred from agglutination of the blood corpuscles by the donor's plasma, but the danger of this is relatively small and it may be disregarded at an advanced post."

In spite of the apparent reliability of our best methods for matching donor and recipient, evidence steadily accumulates to the effect that these in vitro reactions are occasionally wrong. Thus Percy,[4] using a slightly modified Kimpton-Brown[5] tube, reports two transfusions with extensive hemolysis in a total of fifty-four cases, in which careful testing had shown no in vitro hemolysis. Neither of

* From the Medical Clinic of the Peter Bent Brigham Hospital and the Laboratory of Physiology of Harvard Medical School.

1. Moss, W. L.: J. A. M. A. **68**:1905, 1917. For more extended discussion and guide to literature see Moss, W. L.: Bull. Johns Hopkins Hosp. **22**:238, 1911.
2. Lee, R. I.: Brit. M. J. **2**:684, 1917.
3. Presse méd. **26**:193, 1918.
4. Percy, N. M.: Surg., Gynec. & Obst. **21**:360, 1915.
5. Kimpton, A. R.: Boston M. & S. J. **178**:351, 1918. Gives latest technic and refers to previous papers on the subject.

these cases had been transfused previously. McClure and Dunn[6] report one death in 150 transfusions and ascribe it to "improper matching of the blood." Their technic for matching is reliable, and one infers another inexplicable reaction. These authors make a comment on gross hemolytic accidents which should be noted:

> In the patients for whom many transfusions have had to be done, it has been observed that it is more and more difficult to find donors whose blood will match that of the patient. A donor may match perfectly early in the series of transfusions and later be found unsuitable. This is probably due to the development of isohemolysins. It is, therefore, most important that the blood be matched before each transfusion.

Our own experience will illustrate a case very much in point.

> A. A., aged 13, with pernicious anemia and sepsis, required transfusion, Dec. 17, 1917. The mother desired to act as donor and was readily placed in Group 2. The patient's serum and cells behaved irregularly but he seemed to fall in Group 1. On matching directly against the mother a slight agglutination of the mother's corpuscles by the son's plasma was seen, the irregularity the son exhibited as a probable member of Group 1. This agglutination was so slight, so long in appearing, and the mother so insistent in her desire to act as donor that transfusion was done, her blood being taken into citrate, the plasma discarded and the red cells washed twice in accordance with technic described later in this paper. No chill followed this transfusion. There was rise in temperature of 1.8 degrees F., which was not appreciated by the patient.
>
> Two days later the patient received a second transfusion of washed red cells from a Group 3 donor. These cells were entirely compatible. No chill, but a rise in temperature of 1.4 degrees followed this tranfusion.
>
> Next day an exactly similar transfusion from another Group 3 donor was given, without chill but with a rise in temperature of 2.4 degrees. Seven days later, and on the eleventh day following the original transfusion from the mother, a fourth transfusion of whole citrated blood was accomplished, using the mother as donor and omitting any effort to match the bloods. There was a severe and immediate chill, with a rise in temperature of 4.2 degrees, extensive hemoglobinuria and hemorrhage from the mucous membranes.

This reaction would have been avoided had there been an examination just prior to the last transfusion. It arose from the original use of slightly incompatible blood and a second employment of the same blood with a reasonable interval for the development of hemolysins. We have not seen a patient change groups under repeated transfusions and see no reason why he should, provided his original grouping and the grouping of his donors is as definite as the tests usually give. The rare cases, such as the one we have cited, where loose laboratory judgment first permitted injection of slightly incompatible blood and then did not insist on another test, should be excluded on the basis of the questionable first test. In such a situation at present we would use Group 4 donors, testing their cells against the patient's plasma and paying no attention to the exact final grouping of the patient.

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6. McClure, R. D., and Dunn, G. R.: Bull. Johns Hopkins Hosp. **28**:99, 1917.

Sydenstricker, Mason and Rivers[7] report two cases tested by reliable methods in which exceptionally severe hemolysis occurred, death being ascribable to it in one case. In both instances several transfusions had been done previously. It is noteworthy that the fatal hemolysis followed a Lindeman[8] syringe-cannula transfusion, and the nonfatal reaction a citrate transfusion. This has nothing to do with the merits of the two methods, but indicates that such reactions depend on inherent difficulties in the blood, which will be displayed no matter

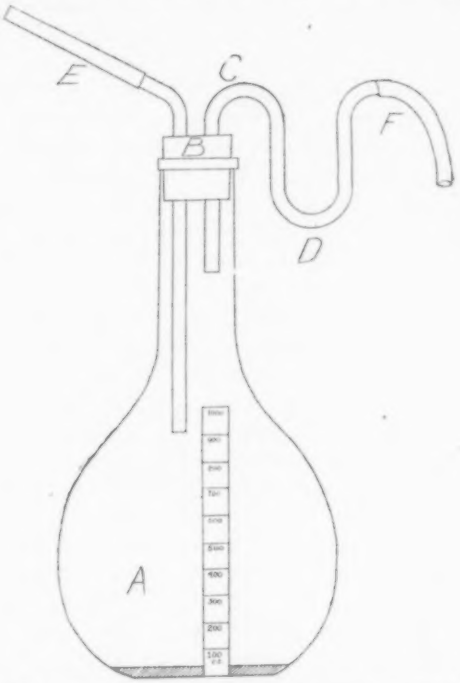

Fig. 1.—Collecting flask containing sodium citrate solution.

how the transfusion is carried out. It is thus apparently inevitable that with the best of testing, rare instances of extensive hemolysis will appear. These cannot be foreseen and have no relation to the method of actual transfusion. They form a foundation in all statistics of reactions, but they are not the type of difficulty with which we are most directly concerned.

The reactions following transfusion are of varied type. There may be slight headache, malaise, urticaria, slight fever, chills and high

7. Sydenstricker, V. P. W., Mason, V. R., and Rivers, T. M.: J. A. M. A. **68**:1677, 1917.

8. Lindeman, E.: Am. J. Dis. Child., **6**:28, 1913.

fever, appreciable hemolysis with hemoglobinuria, acute anaphylactic shock, etc. Out of this list of possibilities the most frequent noteworthy reaction is a rise in temperature, often accompanied by a chill. In our experience few patients undergo a rise in temperature of 2.5 degrees without subjective symptoms of some sort. A chill may precede such a rise, and it may fail to precede a rise of 3.5 degrees, but in this latter case other subjective symptoms will usually be present. In the cases which follow, a 2.5 degree rise of temperature has been the main criterion of a reaction, but since reaction possibilities are varied, this cannot be carried to mathematical finality. There is no single method of interpreting the harm done by injection of foreign protein, and in emphasizing the 2.5 degree rise criterion it is also necessary to mention other features of reactions when they become prominent.

No method of transfusion is reaction-free, and the reactions have been ascribed to a variety of causes, but without effort at experimental analysis. Lindeman[9] is emphatic in his assertion that all febrile reactions following transfusions by his method are due to hemolysis. His substantiation of this statement depends on the fact that he has fewer reactions when hemolysis and agglutination tests of donors have his personal supervision. He presents no direct evidence that hemolysis has occurred in his unfavorable cases. Owing to the fact that reactions probably require very slight hemolysis, too slight to measure by our best methods, Lindeman's omission of direct proof is unavoidable. But it is not possible to hold that the only foreign protein in the blood stream after transfusion must be hemoglobin or, in a broader sense, hemoglobin and the stroma of broken down red cells. The early changes incident on blood coagulation result in the appearance of abnormal proteins, and there is no method of transfusion with the exception of vessel to vessel suture free from the possibility of their production. The conspicuous alteration which blood begins to undergo as soon as it leaves the vessels is clotting. Too frequently the setting of the clot is taken as the beginning and end of the act, but blood kept fluid, grossly apparently quite normal, may still have progressed some distance on the road to coagulation. There is no practicable method for citration of the large quantities of blood needed for transfusion which certainly prevents the early changes of coagulation. The methods in use simply arrest the process. The truth of this statement will be testified to by many who have used the citrate method with complete coagulation of the blood at a critical point in the transfusion. Similarly with the paraffined tubes of Kimpton and Brown,[5] it is necessary to make the transfer before clotting spoils the operation, and here again there will be very few who have not occasionally lost

9. Lindeman, E.: J. A. M. A. **66**:624, 1916.

a tube through the final act of coagulation. In like manner the syringe-cannula method[8] even though carried through with the greatest neatness and dispatch must also present partial products of coagulation to the recipient. Abnormalities incident on coagulation vary widely from case to case, depending to some degree on the method and the skill with which it is carried out. They seem to be produced some time before the actual setting of the coagulum. That coagulation is a

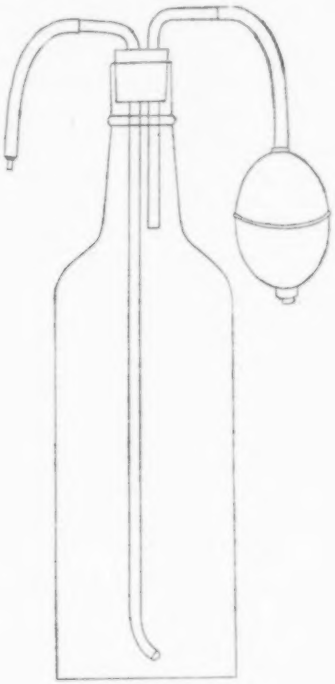

Fig. 2.—Injecting flask.

factor added to hemolysis in causation of reactions has been appreciated as a possibility by Satterlee and Hooker,[10] by Minot and Lee[11] and by Robertson.[12] It is strongly indicated by the many comparative studies on plasma and serum reactions which are well summarized in recent papers by DeKruif[13] and by Janeway, Richardson and Park.[14]

10. Satterlee, H. S., and Hooker, R. S.: J. A. M. A. **66**:618, 1916.
11. Minot, G. R., and Lee, R. I.: Boston M. & S. J. **177**:761, 1917.
12. Robertson, O. H.: Brit. M. J. **1**:477, 1918.
13. DeKruif, P. H.: J. Infect. Dis. **20**:717, 1917.
14. Janeway, T. C., Richardson, H. B., and Park, E. A.: Archives Int. Med. **21**:565, 1918.

Finally, if one makes a series of intravenous injections of a fairly constant type of foreign protein such as typhoid vaccine, he is impressed with the wide difference in reactions presented by different individuals. While in all a sufficient dose will give chills and fever, the severity of the reaction will vary widely. In the case of blood transfusion, the foreign proteins presented are infinitely less toxic than the typhoid bacillus, and there is a consequently wider range of reaction possibilities, a range which could only be presented by high dilutions of typhoid vaccine. Thus, there will be many individuals who are entirely unaffected, there will be others giving minor and negligible reactions, and there will be a final group who react in accordance with our criterion by a temperature of 2.5 degrees or over. The capacity for foreign protein production probably varies in different coagulating bloods, though at present we have no method of directly estimating it. We feel that this possibility is of less importance than the condition of the recipient and would be much interested to see this phase of the question attacked experimentally.

Before approaching our experimental data, it is worth while to sum up the most recent reaction statistics. Lewisohn[15] reports a "chill following the transfusion" in 10 per cent. of seventy-five instances. Meleney, Stearns, Fortuine and Ferry[16] regard the typical reaction as a "chill coming on about one-half hour after the transfusion, a sharp rise in temperature, gradually subsiding to normal in three to eight hours . . ." Their transfusions meriting statistical consideration are as follows:

Citrate Method	No. of Reactions	Per Cent. of Reactions
196	127	64.8
Syringe-Cannula		
73	47	64.4

The reactions are not figured on a 2.5 degree rise in temperature, but comprise all noticeable disturbances in patients under uniform hospital observation following the transfusion. They are significant in the parallelism between citrate and syringe-cannula administration.

Sydenstricker[7] et al. report 100 citrate transfusions with only 17 per cent. of reactions of any sort, from the mildest malaise to the most extreme "chill, stupor and hemoglobinuria." Their observations are on hospital patients under uniform conditions and the figures the lowest given for the citrate method. Lewisohn's[15] 10 per cent. of chills will certainly mean at least another 10 per cent. of distinctly noticeable reactions.

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15. Lewisohn, R.: Ann. Surg. **64**:618, 1916.
16. Meleney, H. E., Stearns, W. W., Fortuine, S. T., and Ferry, R. M.: Am. J. M. Sc. **154**:733, 1917.

Lindeman[9] reports 155 syringe-cannula transfusions with a rise in temperature of two or more degrees in 30 of them, or 19 per cent. Minot and Lee[11] in 92 transfusions "most of them given by the Vincent paraffin tube method," a modification of the technic of Kimpton and Brown,[5] report 37 reactions of varied severity, or 40 per cent. These reactions cannot be analyzed in terms of a definite rise in temperature. Unfortunately, there are no exact observations as to reactions in large

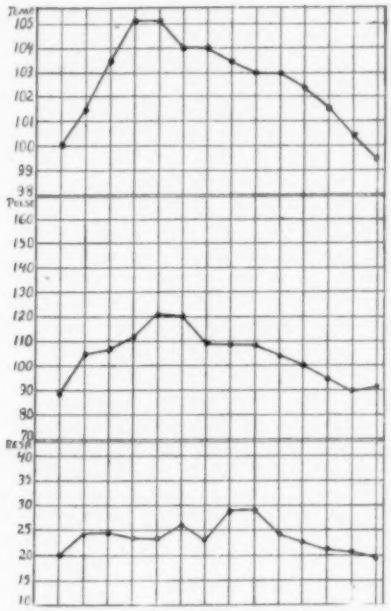

Fig. 3.—M. C.: Pernicious anemia. Group 3. Donor L. F., Group 3. Injection of entire cell content of 600 c.c. donor's blood at start of curve. Abscissae represent half hour intervals; ordinates, temperature, pulse and respiration.

numbers of cases transfused by this method. At the Brigham Hospital, in 19 cases so transfused we have had a temperature rise of 2.5 degrees in 21 per cent.

Our experience with transfusion by the citrate method gives a larger number of reactions than are reported elsewhere. Out of 83 transfusions we have had a rise in temperature of 2.5 degrees 50 times, or 60 per cent., and of this number 39, or 47 per cent., have had chills. These figures include all the transfusions of whole citrated blood which have been done by different individuals at the Brigham Hospital. In order to test the matter further we have done 17 such

transfusions, using the most extreme care in our effort to reduce the reaction percentage. In these transfusions there were 9 rises in temperature above 2.5 degrees, or 53.3 per cent., and 7 chills, or 40 per cent.

It is impossible to summarize these very discordant reports. In totality it would seem that both the syringe-cannula and paraffined tube methods offer fewer reactions than the citrate method. We should count ourselves fortunate could we reduce reactions from transfusion of whole citrated blood to 45 per cent., but it is proper to note that there would be no missed reactions in this group since we have demanded very rigorous post-transfusion observation. We have seen temperatures rise 3 degrees and become normal in three hours, and such reactions will escape notice if frequent observation is not the rule.

TECHNIC

1. *Methods of Recording.*—It is our custom to take temperature, pulse and respiration at the time of transfusion and at half-hour intervals afterwards until the temperature has returned to its original level or given positive indication of soon reaching it. We have seen a number of 2.5 degree rises in temperature accompanied by inconspicuous malaise which would have been missed by ordinary morning and evening temperature taking. For routine work such close following is unnecessary, but for analyzing reactions it is imperative and will, we are sure, disclose many unsuspected increases in temperature.

2. *Methods of Transfusing and Handling Blood.—A.* The Citrate. Four different specimens of sodium citrate have been used, one of unknown origin and probably "commercial" in grade, two from well known firms certified as C.P., and a fourth made especially for us in the Boylston Chemical Laboratory of Harvard University, recrystallized a number of times and probably as pure a product as can be obtained. The number of reactions resulting from all these specimens is the same. There is no peculiarity inherent in certain specimens of sodium citrate which can cause trouble.

B. Citration. A variety of methods has been used and in our hands none of them reduces the number of reactions. The cleanest and best seems as follows. A one liter Florence flask, A (Fig. 1), containing 35 c.c. of 8 per cent. sodium citrate is stoppered with gauze and autoclaved for fifteen minutes at 15 pounds. This does not alter the reaction of the citrate or its ability to prevent coagulation. A rubber stopper, B, carrying two glass tubes is boiled together with a No. 19 needle and the rubber tubes, E and F. E delivers blood to the flask and should not be more than 5 inches long since it is desirable to eliminate as much contact of blood and rubber as is possible. F allows the operator to exert suction. This apparatus avoids stirring the blood and contact with the air. It delivers the blood through the long glass tube directly into the citrate without dripping slowly down the sides of the flask, and the suction facilitates collection very markedly. Thirty-five c.c. of 8 per cent. sodium citrate should keep fluid 1,400 c.c. of blood. It gives the patient 2.8 gm. of citrate, a dose far below the dangerous limit. Such citration insures a strength great enough to restrain coagulation in the 800 or 900 c.c. of blood ordinarily withdrawn, and after repeated observations we are assured it causes no hemolysis within three hours' time. Citration has also been accomplished by using 2.5 per cent. sodium citrate in freshly distilled water in the proportion of 10 c.c. to 90 c.c. of blood, and a 2 per cent. solution in the same proportion. These alterations do not

change the frequency of reaction occurrence. Transfusions have varied in amount from 250 to 950 c.c. We have observed no definite connection between amount of transfusion and extent or frequency of reactions.

C. Injection. The apparatus in Figure 2 is used. It is convenient to clean and carry about and brings the blood in contact with a minimum amount of rubber. A fine needle, No. 21, is thrust into the recipient's vein and with blood flowing freely connection can be made and the donor's blood driven in. Robertson[13] has recently pictured a combination removal and injection apparatus very convenient for emergency work. As routine, however, we believe the blood should be filtered before the final injection. This is readily accomplished by filtration from our collecting flask into the injecting flask, an old,

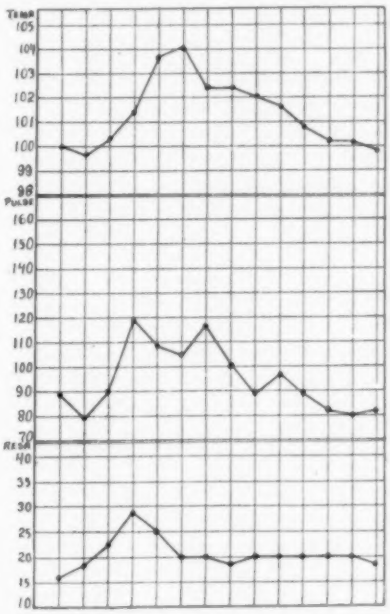

Fig. 4.—M. S.: Chronic nephritis and hypertension. Injection of entire cell content of 700 c.c. own blood at start of curves. Abscissae represent half hour intervals; ordinates, temperature, pulse and respiration.

fine mesh, sterile towel being used. All glassware, both for collection and injection, is cleaned in hot tap water and rinsed with fresh glass distilled water before being tied up for sterilization. Needles and short delivery tubes have been boiled in tap water and shaken dry before use. For all solutions coming in contact with blood, glass distilled water has been used, but flasks containing sterile citrate have frequently stood some days before using. As will be seen later, all salt solution used for direct injection has been made from water distilled and sterilized on the day of use. While it is probable that chills would be induced by 100 c.c. injections of old citrate, our quantities have been 25 or 35 c.c., except in a moderate number of instances when precaution has been taken to have fresh material.

EXPERIMENTAL

1. Does the cause of the reaction reside in cells or in plasma?

At the outset we felt confident that the plasma would be found at fault, but this is far from being the case. Citrated plasma, thoroughly freed from all formed elements by prolonged high speed centrifugalization or by porcelain filtration is singularly nontoxic, contrasting markedly with serum in this regard.

The following transfusion repeated four times on three different patients with pernicious anemia, two of whom were subsequently transfused without reactions by other methods, illustrates the beginning of the answer to Question 1.

Patient M. C., Group 3. Donor L. F., Group 3. Donor bled 600 c.c. into 35 c.c. of 8 per cent. sodium citrate at 11:15 a. m. Blood at once centrifugalized at high speed, the plasma pipetted off and the *entire* cell content washed twice with *freshly made* and freshly sterilized physiologic sodium chlorid solution (0.85 per cent. NaCl). This washing was accomplished in 250 c.c. milk bottles in a large high speed centrifuge, with every precaution as to sterility. The plasma was discarded and the cells brought up to 300 c.c. volume with salt solution and injected into the patient at 1:23 p. m. A characteristic and severe reaction followed. (Fig. 3.)

This experience was repeated on L. T. and T. M., who had never been transfused previously, save that four washings were used instead of two, and in all cases the reactions were identical. Not that there was no attempt to separate the different formed elements of the blood. The injection contained washed red cells, white cells, plates and salt solution, and some one or several of these elements caused the reaction.

In which of the cellular elements does the reaction reside? Defibrination completely eliminates blood plates and markedly reduces the white count. We have done transfusions with washed red cells from defibrinated blood and have markedly reduced our reactions. A full discussion of these transfusions appears later and is mentioned here simply to indicate that salt solution, red cells, white cells in greatly reduced numbers (a reduction to about one-third is our ordinary experience in defibrination) and no plates make the most perfect blood for transfusion we have used. It is sufficient to wash these cells twice. We have not been able to get any satisfactory plate-free, leukocyte-rich preparations for use. Whether one makes the separation by means of defibrination or by means of differential centrifugalization, the plates and white cells are eliminated together, but we have seen enough fairly high white counts in our defibrinated washed cell transfusions to be ready to consider the white cells very slightly at fault, if at all.

Thirty transfusions have been done taking the blood into citrate, centrifugalizing slowly for five minutes, removing the plasma and washing twice. At the termination of the last two centrifugalizations

carried on at highest speed, all "buffy" material was removed from the red cells. Such technic reduces the white cell count below half and plates are found with the greatest difficulty. It is impossible to consider such preparations plate-free, but the number of plates left is certainly exceedingly small. In this series, eleven, or 40 per cent., have had the 2.5 degree rise in temperature and six, or 20 per cent., have had chills. We have reduced the number of reactions as compared with whole citrated blood but are still far from eliminating the difficulty; indeed, our percentage is noticeably worse than many observers have reported with whole blood. As far as is known, blood plates exist to aid in blood coagulation, and to perform this function it is necessary for them to begin to change as soon as blood is shed. Wright and Minot[17] have recently called attention to a definite type of platelet metamorphosis. Citration seems to prevent this type of change quite effectually. We have repeatedly examined plates in citrated plasma and are impressed with the extraordinarily small amount of agglutination which takes place. In no way can one obtain more beautiful plate preparations than by staining smears of such plasma with Wright's stain. Since the plates are the principal formed elements eliminated in this type of transfusion, it is natural to look to them for some share in reaction causation but the absence of any particular morphologic change, coupled with the following experiences, indicate that these elements are only partially to blame. We have made ten injections of platelet suspensions with three, or 30 per cent., of 2.5 degree reactions and two chills. These suspensions have been made in a variety of ways and no method of handling which could in any way imitate the condition of the plates in whole citrated blood has resulted in a uniformly toxic product. The conclusion is thus forced that these formed elements are contributors to the total number of reactions but by no means the sole cause. The type of agglutinative metamorphosis pictured by Wright and Minot[17] does not necessitate toxicity. Injections with this change present have been innocuous and injections containing the plates as isolated structures, morphologically normal, have caused reactions. Two cases of true hemophilia have been treated by combined plasma and plate injections without reaction and with prompt cessation of bleeding. In these instances the washed red corpuscles have been introduced next day. In ten transfusions the citrated plasma has been removed, cleaned of formed elements by one hour to one and one half hours of high speed centrifugalization, the red cells washed twice with thorough removal of "buffy" coat, the cleaned plasma and washed red cells mixed and injected. There have been four, or 40 per cent., of 2.5

17. Wright, J. H., and Minot, G. R.: J. Exper. Med. **26**:395, 1917.

degree reactions and two chills. The addition of cleaned plasma to washed corpuscles and salt solution gives a reaction percentage identical with that obtained from washed corpuscles and salt solution alone.

2. Is the reaction due to some unsuspected factor against which we do not test our donors?

In an effort to answer this question the following transfusions have been done.

M. S. Chronic nephritis and hypertension. Bled 700 c.c. into 25 c.c. 8 per cent. sodium citrate. Plasma discarded. Entire cellular content washed four times, brought to 700 c.c. volume with physiologic saline solution and reinjected. A characteristic reaction followed (Fig. 4).

This result was repeated on J. M., chronic arthritis, and these two cases of plasmapheresis[18] in which the *entire cellular content* had been washed and returned to the same individual are identical with the transfusions containing washed whole cell content mentioned earlier in this article.

It is significant that out of six instances of washed whole cell content injection we have had six chills and pronounced febrile reactions, all well above 2.5 degrees. In two cases of chronic arthritis under treatment with intravenous typhoid vaccine we have withdrawn 550 c.c. of blood into 25 c.c. of 8 per cent. sodium citrate, have filtered this blood into an injecting flask and returned it to the same individual. In one instance there was no reaction whatsoever; in the other there was a rise in temperature of 1.2 degrees, but nothing appreciable to the patient.

Finally, with Dr. J. P. O'Hare, we have done plasmapheresis in chronic nephritis eighteen times, taking the blood in citrate, washing twice and returning the red cells freed as far as possible from plates and leukocytes. The technic corresponds exactly with that used in the transfusions of washed red cells from citrated blood. A detailed account of this work appears in another article. Here it is of interest because not one of these patients had a chill or a rise in temperature of 2.5 degrees. The majority reacted slightly, and in four cases there was a rise above 2 degrees. All were cases of advanced chronic nephritis, and while such individuals may be less reactive than the anemic cases making up the bulk of our figures, the results would seem to indicate that handling of perfectly compatible red cells with minimal exposure to citrate does not induce alterations of marked grade. A further consideration of these cases appears in the final discussion.

3. Does citration alter the erythrocytes so as to render them more susceptible to hemolysis when injected?

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18. Abel, J. J., Rowntree, L. G., and Turner, B. B.: J. Pharmacol. & Exper. Therap. **5**:625, 1914.

Two sets of transfusions bear on this point. In fifteen instances we have taken the blood from donors into our ordinary collecting flasks and have defibrinated at once by shaking with glass beads. This removes all the plates and reduces the white cell count to about one-third. The red cells have been centrifugalized off, washed twice, brought up to original volume with physiologic sodium chlorid solution and injected. These transfusions have given three, or 20 per cent., reactions of 2.5 degrees with one chill, decidedly better figures than we can obtain by any citrated transfusion. They may be contrasted with nine transfusions in which all procedures have been identical except that 25 or 35 c.c. of 8 per cent. sodium citrate have been added to the final red cell suspension one or two hours before injection. In these cases we have had four, or 44 per cent., reactions above 2.5 degrees, with two chills. It would thus seem that the mere addition of a normal dose of citrate to red cells develops slight abnormality. We have been unable to detect the nature of this change. Many histologic examinations of citrated blood and of washed citrated red cells have failed to disclose any abnormalities. As far as whole citrated blood is concerned, this has been the experience of others.[18] When the fragility of red cells, taken from defibrinated blood to which citrate has been added in concentration comparable to that used in transfusion, is compared with cells from the same blood which have not been subjected to citrate, it is frequently but not always found that the citrated cells hemolyze more readily. In our experience the reaction has never gone the other way, provided the conditions of citration are kept comparable to those used in transfusion. The two sets of tubes are identical or the citrated cells, as is more frequent, show greater hemolysis. But this observation does not enable us to predict when reactions will occur. We have seen a pronounced increase in fragility of our red cells exposed to citrate and no reaction whatsoever on the part of the recipient. The increase in fragility simply indicates that exposure to sodium citrate tends to produce abnormal red cells. Clowes[19] has called attention to the similarity of sodium chlorid and sodium citrate in their general effects on protoplasm. The difference between the salts is a function of the anion and the citrate is infinitely more toxic than the chlorid no matter what the test material. It is of further significance that ionized salts of calcium such as calcium chlorid readily neutralize or counteract the effects of both sodium chlorid and sodium citrate. If these facts are applied to the question of transfusion of citrated blood the following considerations are developed. In collecting the blood into sodium citrate care is taken to have a sufficient excess to abolish thoroughly all possibility of

19. Clowes, G. H.: J. Physical Chem. **20**:407, 1916.

calcium effect in bringing about coagulation. It is of prime importance that calcium be eliminated entirely, and in some way, not thoroughly known, the addition of sodium citrate does thoroughly remove ionized calcium. Conditions are at once favorable for the action of the sodium citrate and, indeed, of the sodium chlorid on the red blood corpuscles. What the nature of this action may be we cannot say. The increase in fragility which we have mentioned indicates that some change occurs. Robertson[12] has used 160 c.c. of 3.8 per cent. sodium citrate in his recent work in France. The figure is chosen because it gives with the 500 or 800 c.c. of blood usually taken an osmotic pressure approximating that of 0.85 sodium chlorid and isotonic for the red corpuscles. While this may be a wise precaution, it does not meet the true biologic difficulty with the citrate. Citrated blood such as we have used does not contain corpuscles damaged by abnormal osmotic relations, but corpuscles damaged by sodium citrate operating as a specifically harmful substance. Unfortunately, no studies exist giving data exactly analogous to those for which we have suggested a necessity. There are many comparisons between the hemolytic effects of various salts in equimolecular solutions. Thus, recently, Chauffard and Huber[20] have compared the hemolytic effect of Ringer's solution, sodium chlorid, magnesium chlorid, etc., taking care to have an identical freezing point throughout their entire series of comparisons. They have again demonstrated that these salts do not act by virtue of alterations in osmotic pressure, but through individual differences, the final nature of which are unknown. What is called for in the solution of such a question as arises here, is comparison of the fragility of red corpuscles by means of one type of solution, such as varying concentrations of sodium chlorid, after immersion under constant conditions in a variety of equimolecular solutions of sodium citrate, sodium acetate, sodium oxalate, etc.

It is is clear at once that, provided sodium citrate produces abnormality in red cells, rendering them more suspectible to hemolysis, we cannot be surprised at the failure of sodium chlorid, a substance acting in the same way, but to a less degree, to wash out the effect of citrate. Thus, in our thirty transfusions of corpuscles taken into citrate and washed in physiologic sodium chlorid solution we have had 40 per cent. of reactions. If after the second washing calcium chlorid is added to the salt solution, making a concentration of 0.025 per cent. calcium chlorid analogous to Ringer's solution, and in this a third washing and the final suspension of the corpuscles for injection is carried out, there is still no reduction in the number of reactions in transfusions. It would seem, therefore, that the change

20. Chauffard, A., and Huber, J.: Presse méd. **26**:141, 1918.

induced by citrate in the protoplasmic system represented by the red corpuscles is irreversible by calcium within the time of exposure used — from one to three hours.

DISCUSSION

Our interest in this question arose from a desire to get information on the best type of blood modified for injection which could be prepared. If it were true that the plasma from blood which has passed through the manipulations necessary for collection contained all or most of the toxic products involved, the problem would be simple. A nontoxic blood, always excepting detectable disasters from agglutination and hemolysis, could be obtained by simple washing of compatible red cells. Unfortunately the reverse is true, plasma being nontoxic and cellular elements varying in toxicity. We believe that one element in the appearance of this toxicity resides in the plates, which begin to become abnormal as soon as they leave the vessel. The grade of abnormality of these elements depends to some degree on the extent and skill of the handling which they are compelled to undergo in varied methods of transfusion. On this phase of reaction causation will depend the fact noted by others that dexterity in any type of transfusion means fewer reactions. The change which the plates undergo is not evidenced by any characteristic morphology, nor is it possible to get a high percentage of reactions from plates alone. But here one is faced by two difficulties. The first is the impossibility of getting all of the plates out of a blood collected for transfusion and manipulating them so that injection is safe. Their extraordinary agglutinative power makes it very difficult to obtain fair sized injections of washed plates which are free from large masses. Second, the thromboplastic material which the plates present makes a proper degree of hesitation necessary, since intravascular coagulation is too dangerous an outcome to be viewed lightly. The platelet injections which we quote are the result of a gradual increase in dose and the reactions obtained have required the maximum yield of from 500 to 650 c.c. of citrated blood. Such injections always contain many leukocytes, but our experiences have never pointed to these elements as important factors in the development of toxicity. Finally, in regard to the plates and leukocytes, it should be noted that we have no tests for agglutination or lysis of these elements, and the possibility that unexpected incompatibilities may reside in them should be borne in mind.

If we consider that our efforts to remove plates from citrated blood by differential centrifugalization have been successful, and histologically they are so, it is evident that red cells exposed to citrate in collection are more apt to cause reactions than those taken from

defibrinated blood and subjected to a comparable amount of washing, 40 per cent. of reactions occurring in the first case and 20 per cent. in the second. If to these last cells citrate is added as the single difference in their treatment, the reactions again become 44 per cent. Citration, therefore, seems to harm red cells and possible direct evidence for this exists in the occasional promotion of fragility by the substance. The indication is that hemolysis contributes a certain number of reactions but that this hemolysis is too slight to be detected by direct methods.

The results with plasmapheresis confirm our transfusion experiences insofar as injection of washed whole cell blood is concerned. There is, however, a wide discrepancy between plasmapheresis using citrated blood with platelet elimination, and transfusion embodying similar maneuvers. In these latter there is no possibility of cellular incompatibility so slight as to be unrecognized. If citration results in greater cellular fragility, it will do its minimum of harm in these injections, whereas in transfers between different individuals it is possible that an influence which promotes fragility even very slightly may result in a degree of hemolysis of sufficient magnitude to cause reaction. We have suggested that the cases of chronic nephritis which have been subjected to plasmapheresis may be less reactive than are the anemias. Without transfusion of a series of these cases no definite statement can be made. Certainly in our hands cases of secondary anemia from simple bleeding or from bleeding plus carcinoma have reacted as freely as have the primary anemias. The final factor of the individual in these reactions is as yet almost unknown. The tests for agglutination and hemolysis present a certain amount of valuable qualitative information in this direction, but beyond these we cannot go.

SUMMARY

1. Reaction figures are given for various methods of transfusion. Those for the citrate method are widely divergent, but in the main exceed the results obtained by paraffin tube or syringe-cannula methods.

2. A rise in temperature of 2.5 degrees F. is suggested as the best criterion of a reaction, and the technic used in citrate transfusions is discussed.

3. The number of reactions bears no constant relation to method of citration or specimen of citrates.

4. Citrated plasma free from formed elements is nontoxic in compatible individuals.

5. Washed whole cell content of blood is uniformly toxic, whether injected into the individual from which the cells have been removed

(plasmapheresis) or into different individuals who have been tested and show no agglutination or hemolysis of red cells.

6. Removal of the plates and two-thirds of the leukocytes from citrated blood by differential centrifugalization with final suspension of the red cells in freshly made sterile physiologic sodium chlorid solution has resulted in 40 per cent. of reactions in thirty transfusions.

7. Removal of the plates and a comparable number of white cells by defibrination, with thorough washing and suspension of the red cells in similar saline solution has resulted in 20 per cent. of reactions in fifteen transfusions.

8. Addition of citrate from one to two hours before injection to transfusing blood so prepared has resulted in 44 per cent. of reactions in nine transfusions.

9. While the totals of the different types of transfusions which have been employed are not large, it appears that three elements make up the final total of reactions in citrate transfusion: (*a*) very rare gross incompatibilities which escape in vitro detection; (*b*) changes in the plates, part of the process of coagulation; (*c*) direct action of the sodium citrate on red cells promoting hemolysis.

THE CHRONIC FORM OF MENINGOCOCCUS MENINGITIS *

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Cerebrospinal meningitis with a prolonged course presents two varieties. In one we observe only recurrences or episodic meningeal manifestations. Here the patient during the intervals appears to be in perfect health. Nevertheless, suddenly or insidiously the symptoms return either in a slight or grave form. The intervals may last either a few days or a few weeks.

The other variety which is the subject of the present contribution, concerns cases of cerebrospinal meningitis the evolution of which is continuous. While in the literature are mentioned a few such isolated cases under different captions, nevertheless their chief characteristic features have not been sufficiently emphasized by various authors with the exception of Robert Debré, pupil of Netter. In 1845 Tungel[1] for the first time speaks of disturbances in the brain tissue proper in the course of a prolonged case of cerebrospinal meningitis. The next two earliest records we find by Rilliet[2] and Merkel.[3] They both speak of chronic hydrocephalus following a very prolonged course of cerebrospinal meningitis. Since then a number of records have accumulated in the German, French and American literatures indicating various isolated complications in the central nervous system. The present contribution is based on a study of ten cases of meningitis of the meningococcus type kept under observation for several years. Eight of these cases came to necropsy, so that in the largest majority of the series, clinical as well as anatomic data were available for consideration. The cases are very briefly as follows:

REPORT OF CASES

Case 1.—M. S., man of 27, mason, had a typical attack of epidemic cerebrospinal meningitis. The meningococcus was found. The spinal fluid presented polynucleosis and increase of albumin. The acute stage lasted eight days. There was a remission, but some rigidity of the neck remained. There was an exacerbation during five days, with remission again and a gradual deterioration, and muscular atrophy with a paretic condition, ataxia of all extremities,

* Read at the meeting of the American Neurological Association, Atlantic City, N. J., May 9-10, 1918.

1. Tungel: Cited by Ebstein, Deutsch. Arch. f. klin. Med., 1908, **93**, 241.
2. Arch. Gén. de Méd., 1847.
3. Merkel: Deutsch. Arch. f. klin. Med., 1866, **1**.

knee jerk diminished, decubitus, pain in the legs, mental dulness, deafness; spinal fluid almost clear, with mononucleosis. The patient lived five months. Necropsy report is given later.

Case 2.—O. O'B., man 31, a laborer, had the usual acute onset. He had nine exacerbations during six months. In the intervals he developed amyotrophy with contracture of the limbs in flexion. There was decubitus in the occipital region, incontinence of sphincters; no spinal fluid was obtainable. There was deafness, palsy of internal recti in both eyes, mental hebetude, an occasional delirious state. The patient died in convulsions. No necropsy.

Case 3.—M. S., girl, aged 25. Typical form. The patient had five exacerbations during ten months. There was amyotrophy with paresis of the lower extremities, contracture in flexion, incontinence: reflexes (tendon) diminished; deafness; impaired vision; palsy of left external rectus. Very little spinal fluid was obtainable, which was very slightly turbid. There was mononucleosis. The meningococcus was not found. The patient assumed a stuporous state. Necropsy.

Case 4.—B. G., man aged 25, a watchmaker. Typical form. He had three exacerbations in four months; amyotrophy; paresis of all limbs; tendon reflexes much diminished; decubitus; pain in the lower extremities; deafness; vision impaired; incontinence; mental hebetude. Very little spinal fluid was obtainable on lumbar puncture and no meningococci were found. No necropsy.

Case 5.—S. D., a man aged 29 years. Typical form. He had six exacerbations in thirteen months. The condition following each successive exacerbation was worse than the preceding. All the characteristic symptoms were pronounced, namely, amyotrophy, decubitus, contracture in flexion; knee jerks plus; deafness; palsy of left external rectus. No spinal fluid could be obtained after several attempts. Mental dulness. Necropsy.

Case 6.—A. G., a boy, aged 19. Typical form. He had three exacerbations in six months: amyotrophy with flexion contracture of the legs was so pronounced that the knees touched the chin. The knee jerks were lost. There was incontinence of urine and feces and decubitus; spinal fluid very slightly turbid; mononucleosis; meningococcus not found; mental hebetude. Necropsy.

Case 7.—C. S., a man, aged 32. Typical form. Had five exacerbations in ten months. Amyotrophy with contractures of lower limbs; decubitus; mental dulness, with periods of improvement; incontinence; spinal fluid clear, no meningococci, but mononucleosis; palsy of internal recti in both eyes; mental dulness. Necropsy.

Case 8.—A. P., girl, aged 22. Typical attack. She had three exacerbations in five months. Amyotrophy with mild contracture of legs and paretic condition; knee jerks diminished; incontinence; deafness; impairment of vision; mental hebetude, but at times total lucidity; delirium with each exacerbation and a mild confusional state at times in the intervals; spinal fluid clear; mononucleosis. Died suddenly. Necropsy.

Case 9.—J. O'H., boy, aged 13. Typical form. He had five exacerbations in two and a half months. Amyotrophy with contracture of legs; incontinence; decubitus; impairment of vision; spinal fluid clear, but with a yellowish tint; mononucleosis; mental dulness, with occasional mild delirium. Necropsy.

Case 10.—S. H., girl, aged 7. Typical form. Six exacerbations in four months; amyotrophy with contracture of legs in extension; extreme emaciation; incontinence; mental dulness with occasional complete lucidity; spinal fluid clear, with a yellowish tint; mononucleosis; impairment of vision; palsy of the right external rectus; died in convulsions. Necropsy.

A detailed analysis of these cases brings out the following important anatomic and clinical facts.

Pathology.—The anatomic-pathologic findings in the eight cases that came to necropsy are as follows: The condition of the meninges could be characterized as one of diffuse pachymeningitis: irregularly distributed patches of thickened membranes could be seen, especially along the larger blood vessels and mostly at the base of the brain and near the cerebellum. The blood vessels appeared congested. Purulent areas were not frequent but if they occurred, were seen particularly at the level of the chiasma. Adhesions between the dura and the pia-arachnoid, as well as between the latter and the cortex, were occasionally seen, but more frequently the pia could be detached from the cortex without tearing the tissue of the latter. In several of the cases small cystic collections were found at the level of the pons.

The brain itself appeared on palpation somewhat softer than normal. It was due to dilatation of the lateral ventricles. In all the eight cases they were filled with considerable quantities of turbid fluid, and in two of these cases it was frankly purulent. There was apparently little or no communication between the various ventricles, as they were all overfilled with fluid. The histologic examination revealed in the meninges the usual findings of a chronic inflammatory state, namely, large masses of fibrous tissue with leukocytic infiltration of the walls of the blood vessels. In the cortex besides an edematous condition there was also infiltration of the blood vessels and proliferation of neuroglia. A certain degree of chromatolysis was evident throughout.

In the spinal cord, thickening of the meninges with meningeal adhesions in isolated areas were seen over the posterior aspect of the cord and especially at the level of the cervical and lumbar segments. The spinal fluid was less turbid than that of cerebral ventricles and sometimes very clear. The tissue of the cord suffered only at the periphery close to the altered meninges. Leukocytic infiltration with dilatation of some blood vessels was observed. Of special interest are the alterations found in the roots, more in the posterior than in the anterior ones, and in the nerve trunks. Leukocytic infiltration of the latter's blood vessels and actual degenerative changes in the nerve fibers were in evidence. Specimens were taken at the level of the cervical and lumbosacral segments. Portions of individual nerves of the brachial and sacral plexuses were examined. Chromatolysis was present in the posterior spinal ganglia.

The cerebrospinal fluid deserves special mention. I have already spoken of the appearance of this ffuid and of its greater turbidity in the ventricles than in the subdural space of the spinal cord. In the latter I found it to be somewhat yellowish in two cases, but in the majority of the cases it was clear. Other writers observed the change of turbid to clear fluid in prolonged cases, although it is not so in every

case. Hajek,[4] for example, observed during an epidemic of cerebro-spinal meningitis in Milan clear fluid at first, then later purulent and then again clear. Netter and Debré[5] in their extensive studies also report such occurrences in rare cases. Albumin was found in the earlier stages of the disease in large quantities; in the later periods, three and four months after the onset, the albumin content was negligible. The polymorphonuclear cells of the early periods disappeared and mononucleosis was almost exclusively found weeks later. The same condition was found with regard to the meningococci. While they were abundant in the beginning, their number gradually became smaller and smaller, and several weeks later they were very rare. Cultures made in three cases from the spinal fluid, respectively six, eight and nine weeks later, failed to reveal the presence of the micro-organism. The disappearance of the meningococcus ran parallel with the clearing up of the spinal fluid. Other authors, however, reported the occasional presence of the micro-organism in perfectly clear fluids. Hajek[4] made daily punctures in a child of 3 during eight days; the spinal fluid was invariably clear and still a few meningococci were found, but curiously enough they were extracellular instead of being included in the cells.

Clinical.—The clinical picture based on the ten cases presents the following chief characteristics: The onset shows nothing unusual worth mentioning. The initial symptoms gradually subside and to all appearances recovery is expected, but close observation reveals that it is only a remission, as there are still physical and mental manifestations sufficiently evident to consider the patient ill; some rigidity of the neck and of spine, some difficulty of walking are all present. Mentally the patient shows a diminution of attentive power, of the mnemonic faculty and of general intelligence. The remission may last various times. In my cases from nine to twenty-one days. In the midst of the apparent amelioration of the symptoms there is suddenly or rapidly a reappearance of the manifestations characteristic of the acute stage: the foregoing mental and physical symptoms become accentuated and fever is added. Again, this symptom group will gradually subside for a period of one or several weeks, but nevertheless will not totally disappear. The curve of accentuation and amelioration of the condition may repeat itself an indefinite number of times. Gradually one observes the development of special disturbances which are so constant that they may be considered as characteristic of the

4. Hajek: Pediatria, January, 1909.
5. Netter and Debré: Bull. Soc. méd. d. hôp., Paris, July 29, 1899, and May 11, 1900.

chronic form of meningococcus meningitis. They are seen in the motor, sensory, trophic and psychic spheres.

The most conspicious change takes place in the state of *general nutrition* and especially in that of the musculature of the body. It is diffuse and not confined to any one area. The limbs become extremely thin, the usual roundness about the shoulders and hips disappears and the bony processes are very conspicuous. The subcutaneous fat disappears and the bones appear to be covered with a thin and wrinkled segmental covering which is dry and without the usual elasticity. Erythematous and herpetic disorders readily develop. Decubitus in the sacral, in the occipital (one case) regions and also on the heels are common.

Motor disturbances are especially evident in a body affected with a marked amyotrophy. The neck becomes more rigid than formerly. The limbs are in a state of contracture and in flexion and thus the patient remains immobile. In Case 6 the contracture and rigidity were so pronounced that the patient was all doubled up, with his knees almost reaching the chin. In less contracted limbs (Cases 2, 3, 5, 9 and 10) movements are possible, but there is a distinct paretic condition with diminished reflexes, and the movements remind one of those which are observed in cerebellar affections, namely, ataxia or awkwardness. In only one case (5) the knee jerks were increased, but there was no ankle-clonus and no toe phenomenon. In all the other cases the knee jerks were diminished, and in Case 6, with extreme contracture, the patellar tendon reflex was not obtainable.

Sensory disturbances are observed chiefly in the subjective field. Rigidity, with pain in the neck, spontaneous paroxysms of pain, especially in the cervical and dorsolumbar regions radiating down in the limbs, severe headache, are almost constant. Hyperesthesia is a striking symptom. The least touch or change of position provokes pain. The special sensorium is not infrequently invaded. In every one of my cases there was more or less involvement of the hearing, in five there was impaired vision, although the ocular fundi remained intact. In four of the latter there remained some involvement of the eye muscles, which became paralyzed during one or another recrudescence of the original symptoms accompanied by fever.

In all the ten cases the *sphincters* of the bladder and rectum were involved; during the entire course of the disease incontinence was present, with this difference, however, that during the intervals between the periods of recrudescence, it was less marked. Nevertheless, toward the end, namely, many weeks after the onset, when no more phases of acute symptoms occurred, the incontinence remained unaltered and more and more disturbing.

The psychic status of the patients is characterized particularly by a general intellectual hebetude. The degree of the latter varies from one patient to another and in the same patient at various periods of his disease. They all appeared indifferent, seemingly unable to understand when spoken to. Questions have to be repeated before they show evidence of grasping their meaning. The apathy and indolence may be extreme. The emotional sphere is almost obliterated. When pain is brought on by a change of position, or by a movement of a limb, the facies will not exhibit much suffering. On the other hand, when the same manipulations are made during a period of amelioration of the mental condition, the suffering of the patient is intense. In Cases 3, 5, 7, 8 and 10, there were phases of considerable improvement in the intellectual faculties without a corresponding improvement in the somatic disturbances. Such an improvement may go even as far as to present complete lucidity (in Cases 8 and 10), but the latter is not permanent, and a return to the debilitating status does not fail to take place. In some cases during the periods of recrudescence, namely, when the symptoms become accentuated and present the same picture as during the acute stage, especially when the temperature rises, a delirious state with confusion and incoherence is observed. It disappears when the acute symptoms subside and the patient returns to the chronic course of his malady. In Cases 8 and 10 a mild delirium was observed two and three times, respectively, not during recrudescence of the symptoms, but without a febrile state and when the trophic disturbances became much pronounced. The delirium is then probably due to the state of inanition or exhaustion.

The *course* of the disease in my ten patients was variable. Some of them had more acute attacks than others. The elevation of the temperature during the latter varied also from case to case. The state of prostration which follows the acute attack differed from one patient to another. But what was common to all cases is that the state of mental and physical exhaustion after the individual attacks were more and more profound with each successive attack; also that the course of the disease is very insidious and that, at least in my cases, the termination was fatal. The longest case lasted thirteen months and the briefest two and a half months. In one case (2) the patient died in convulsions which lasted five hours. There was a status epilepticus, as every five minutes a seizure would occur. Patient 1 died suddenly. All others expired after a gradually increasing prostration.

As to the age, my patients were, with two exceptions, all adults. The two cases were children, of 7 and 13 years, respectively. The 13-year-old patient presented no different features from those of the

adults. In the 7-year-old girl the only peculiarity noticed was contracture of the limbs in extension different from that in adults, in whom flexion was the striking feature.

The *pathogenesis* of the chronic form of meningococcus meningitis was not difficult to determine in view of the anatomic findings. Ventricular dilatation with secondary intracranial hypertension; otherwise speaking, a hydrocephalus as a sequel of a meningococcus meningitis is the chief morbid condition. It will readily explain the chronic psychic state which is so conspicuous in the form of meningitis under discussion. Moreover, the trophic disturbances — the sensorimotor manifestations and the state of the sphincters, which all run a chronic course and are so pronounced — are due to the profound degenerative condition of the nerve roots and of the peripheral nerves. Although a recovery is hardly to be expected under such conditions, nevertheless occasionally such recoveries have been recorded.

Diagnosis. — From a diagnostic standpoint difficulties may be encountered, especially when cases fall under observation weeks or months after the onset. First of all, the spinal fluid at this period of the disease is, as we have seen, more or less cytologically mononuclear. Besides, the meningococcus is not to be found directly or culturally. One may think, therefore, of a tuberculous meningitis. In such cases the precipito-reaction of Vincent with tuberculin may be of assistance. On the other hand, the syndrome of intracranial hypertension, especially when visual disturbances are present, will direct us toward cerebral neoplasm. Finally, the subjective and the objective sensory disturbances, together with the motor manifestations, may make one consider polio-encephalitis, polyneuritis or poliomyelitis. The diagnosis must be based on the ensemble of the various individual phases of the disease.

Treatment.—The character of the lesions in the chronic form of meningococcus meningitis precludes the possibility of obtaining results from any form of treatment. In every one of my cases the lumbar punctures were tried for the purpose of injecting antimeningococcus serum. In some cases very small quantities of fluid were obtained; in one case (5) no fluid at all escaped through the needle on several attempts. The serum was injected into the canal at various intervals, but no favorable results were obtained, a fact which I fully anticipated. The pachymeningitis, various adhesions, absence of communication between the cranial and spinal cavities — are all circumstances which, on the one hand, prevented the spinal fluid from escaping sufficiently through the puncturing needle, and, on the other hand, prevented the injected serum from reaching the ventricles, the main seat of the meningococci. Ventricular punctures with injection of serum into

them are therefore directly indicated, but permission could not be obtained for such procedures in my cases. It is to be presumed that when attempts of this character are made during the early phases of the affection, when the tendency to chronicity is first observed, desirable results could be expected. The chronic form of cerebrospinal meningitis is one of the most serious affections. Since it usually follows the acute form which had not been sufficiently treated with antimeningococcus serum, its recognition is of paramount importance.

1812 Spruce Street.

AN UNUSUAL COMBINATION OF CARDIAC ARRHYTHMIAS OF ATRIAL ORIGIN OCCURRING IN A PATIENT WITH FOCAL INFECTIONS AND THYROID ADENOMATA*

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In view of the growing interest in unusual forms of cardiac arrhythmia and their relation to focal infections on the one hand and to thyreopathic disturbances on the other, it has seemed to us worth while to record a case in which remarkable disturbances of cardiac rhythm were encountered in association with (1) peridental, maxillary and tonsillar infection, and (2) minute adenomata of the thyroid gland; and in which marked improvement followed treatment of the infected foci and partial thyroidectotmy.

A. CLINICAL HISTORY

ANAMNESIS.—The patient, K. I. N., a salesman, aged 51, married, applied for a general diagnostic study on Feb. 11, 1918.

Family History.—Father died at 90, cause unknown; mother died at 47, at childbirth; one brother, older than the patient, has suffered from a nervous breakdown and has had some thyroid trouble; one sister died of thyroid disease; a second sister has never been robust. Married several years. No children.

Previous History.—Aside from the ordinary diseases of childhood and a severe tonsillitis at 20, the patient has been a singularly robust man—active, energetic, and a hard worker. Through force of circumstances he was compelled to undertake remunerative work at the age of 11 and has been hard at work ever since until the onset of the present illness. Some twelve years prior to admission he met with serious business reverses and after that had to double his efforts to try to recoup his losses. He has never had typhoid, pneumonia, rheumatism nor venereal disease. Aside from a partial tonsillectomy in early life, he has never had any surgical operations. Except for overwork, he has lived fairly hygienically. He is a good walker and has always taken calisthenic exercises at home. Beginning at 25, he has used tobacco moderately, averaging six to eight cigars per day; during the past year he has reduced the amount to three mild cigars a day He has been almost a total abstainer from alcohol, taking only occasionally a single drink. He states that he has eaten carefully, has not used tea or coffee to excess, and has not resorted habitually to the use of any drug. There is no history of sexual excesses.

* From the Medical Clinic of the Johns Hopkins Hospital.

Present Illness.—Though for the past three or four years he has suffered from insomnia and for more than one year has been somewhat depressed, getting up in the morning feeling worse than on going to bed, he dates the onset of the present illness to an attack nine months prior to admission, supposed to have been "grippe." He ached all over, suffered from shortness of breath, especially on exertion, had an occasional headache without dizziness, and felt weak. His pulse was rapid; he thinks the pulse rate was from 125 to 130 at bedtime. His voice, he says, seemed unnatural. He consulted a physician, who told him that he had "heart disease." He remained under treatment for some four and a half months, though he was not put to bed. He improved somewhat, but did not feel well, and finally was given a course of Nauheim baths, which, he says, made him feel better and reduced his pulse rate to 75 or 80. He still suffers from attacks of fluttering of the heart, with rapid pulse, and complains that his eyes "feel like lead." He remains depressed though there is no reason that he can see for his depression aside from his physical state. He is no longer worried about business matters for his business is now successful. The depression has, however, not been severe, for he has never had weeping spells or any suicidal ideas. He has been having some trouble with his teeth and is under treatment by a dental surgeon.

Physical Examination.—Gait, attitude and posture normal. Fairly well nourished. Height 5 feet 10½ inches. Ideal weight, 167¾ pounds; actual weight, 157¾ pounds stripped. Musculature well developed. Patient is alert and a little apprehensive. The skin is generally a little pigmented; the lips are slightly cyanotic. The superficial veins over the body are slightly dilated and look bluish. The temperature taken over several days varied between 99.3 and 103 F. Pulse rate variable, often 110 or 120. Blood pressure, on first examination, 145 systolic, 80 diastolic.

Head well formed. Acra fairly prominent. Hair abundant and slightly gray. Trace of icteric tint in the conjunctivae. Eyes distinctly prominent. Von Graefe's sign slightly positive. Slight double arcus senilis. Pupils equal; react promptly to light and accommodation. Ears negative. Many teeth have been extracted—all the lower right molars are gone. Gum margins uneven and unhealthy. Lower left molars gone. Upper incisors and bicuspids replaced by bridge. Hearing, sight and smell good. Slight nasal obstruction, more marked on the right than on the left. Tonsils look suspicious. Movements of the face and tongue normal. Speech not disturbed, except that the voice is rather low-pitched and somewhat monotonous.

The neck is a little fuller than normal and there is very marked pulsation of the vessels of the neck, particularly on the right side. The thyroid gland is slightly, but definitely, enlarged, particularly the right lobe. The isthmus is palpable, and the left lobe can also be felt. There is a slightly nodular feel to the thyroid suggestive of minute adenomata. A slight bruit is audible over the right lateral lobe of the thyroid. No tracheal tug. No cervical rib. No enlargement of the lymph glands of the neck. Movements of cervical spine normal.

Thorax is well formed, symmetrical; expansion good, equal on the two sides. Epigastric angle wide. There is a dilated vein running over the upper right chest, not visible on the left. Percussion sounds normal over the lungs. Breath sounds normal over both fronts and backs. There is rather marked cyanosis of the face and hands and a little clubbing of the finger tips.

The apex beat of the heart is in the fifth space, just lateral from the mamillary line. There is some increase of retrosternal dulness. The left chest is shaken a little with each heart beat. No thrill felt over the heart though the shocks of both the first and second sound can be readily felt at the apex. On auscultation there is tachycardia and a tendency to pendulum rhythm, but no heart murmurs are heard. The heart rate is about the same lying as standing. The heart is obviously a little enlarged on percussion. The abdomen is symmetrical. The spleen is not palpable. The edge of the liver can be just felt below the costal margin at the end of deep inspiration. No

hernia. No abnormal masses palpable in the abdomen. No tenderness over the appendix or over the gallbladder. Rectal examination negative. Left testicle undescended. Sensation normal. No paralyses. Reflexes normal.

LABORATORY TESTS

Blood Examination: Red blood corpuscles, 5,120,000; hemoglobin, 100 per cent.; white blood corpuscles, 9,400.

	Number	Per Cent.
Polymorphonuclears	175	70.0
Eosinophils	1	0.4
Basophils	0	0.0
Small mononuclears	65	26.0
Large mononuclears / Transitionals	9	3.6
	250	100.0

Red blood corpuscles and platelets normal. No abnormal cells seen.

Blood Wassermann Reaction: Antigen A—Cholesterinized human heart—negative. Antigen B—Acetone insoluble lipoids—negative. Antigen C—Plain extract beef heart—negative.

Gastric Analysis.—Thirty c.c. recovered; colorless; free hydrochloric acid, 20; combined acid, 48; total acidity, 68.

Occult blood, 0; lactic acid, 0; microscopically negative.

Stool.—Brown, formed; bile, plus; occult blood, guaiac, 0; benzidin, 0; microscopically negative.

Urine.—Specific gravity, night and day, 1.024 and 1.020, respectively; albumin, faint trace; sugar, none. Microscopically: Moderate number of white and red blood corpuscles. No casts.

ROENTGEN-RAY EXAMINATIONS

Roentgenoscopic of Chest.—Heart somewhat enlarged, lying transversely; diffuse dilatation of aorta; no sacculation; good pulsation throughout. Slight anterior encroachment on retrocardiac space in its inferior portion.

Impression: Diffuse, moderate dilatation of aorta; slight enlargement of the heart.

Roentgenoscopic of Gastro-Intestinal Tract.—Cowhorn, hypertonic stomach; peristaltic waves slightly increased in depth and frequency; duodenal cap not visualized. Flexibility good and mobility normal; no filling defects; pyloric extremity in apposition with hepatic flexure but separable from it by palpation. Hepatic flexure at level of iliac crests; transverse and descending colon negative.

Serial Roentgenograms of Gastro-Intestinal Tract.—Hypertonic, cowhorn stomach in good position; no filling defects; rapid emptying; no six hour retention. Colon in good position; marked cecal stasis.

Roentgenogram of Paranasal Sinuses.—There is very slight clouding of the right antrum; otherwise the paranasal sinuses are negative.

Roentgenogram of Lungs.—The upper thirds of both lungs show some slight clouding, especially in the apical regions. Probably old, inactive tuberculous process.

Teleroentgenogram.—

M. R. (= maximal distance from median line to right margin of heart).. 6.7
M. L. (= maximal distance from median line to left margin of heart).... 9.1
L. (=length of heart shadow).....16.5
Br. (= breadth of heart shadow).....11.9

The heart is rather large both on the right and on the left. It lies slightly transversely. The aortic knuckle is a little dilated. There is some mediastinitis both on the right and on the left, more marked on the right. There are increased shadows due to calcified glands at the roots of both lungs. The bases are both slightly cloudy.

Roentgenogram of Sella Turcica.—No definite outline of the sella is visible.

Roentgenogram of Clavicular Regions.—This gives no evidence of cervical rib; no unusual substernal shadows.

SPECIAL TESTS

Epinephrin Test.—

Time	Pulse	Blood Pressure	
10:35	124	140/85	Dyspnea from climbing stairs; slight tremor of fingers; hands dry.
10:45	104	135/85	Dyspnea much less; pulse slower; quite regular; tremor +; slight moisture of hands.
10:47	...	.....	Epinephrin, 0.5 c.c., hypodermically.
10:49	104	133/80	Tremor +; slight moisture of hands.
10:55	112	125/75	Definite increase of tremor; hands moist.
11:00	112	125/75	Marked tremor; hands moist.
11:05	112	128/80	Marked tremor; hands moist.
11:12	112	130/80	Marked tremor; hands moist.
11:20	108	125/75	Marked tremor; hands moist.
11:35	108	123/70	Marked tremor; hands moist.
11:45	112	126/70	Marked tremor; hands moist.
11:55	108	120/65	Still marked tremor; hands moist; slight arrhythmia.
12:05	112	123/70	Still marked tremor; hands moist; slight arrhythmia.
12:10	150 (?)	120/70	Patient sits up quietly; pulse almost uncountable; marked irregularity in force and rhythm; "palpitation"; marked tremor.
12:15	96	140/80	Still in sitting posture; very little palpitation; tremor well marked. Says "heart often acts that way."

Impression: The marked increase in tremor of the hands, increase in pulse rate and perspiration would indicate some epinephrin hypersensitiveness; the late appearing palpitation with arrhythmia and tachycardia may also be further proof.

Dental Report.—Examination shows an osteomyelitis of the superior maxillary bones, extending to the buccal wall of the sinus. There is also an indurative gingivitis.

Rhinologist's Report.—There is a very definite chronic infection of the tonsils, particularly the left. Both tonsils have been partially removed. Hypertrophied lymphoid tissue in nasopharynx. The nasal septum is deflected, causing obstruction on the right side of the nose.

The right antrum is dark on transillumination. Would advise roentgenogram of sinuses.

In nasal examination no definite evidence of any infection of the sinuses can be made out. No polypi. No discharge to be seen.

Psychiatrist's Report.—A very searching examination of the patient reveals practically nothing but too limited a mental diet, with continuous work, without any recreative interests, a rather shut-in family tendency and at present an impossibility of getting the exercises on which he used to depend—hunting, horseback riding, walking, etc. The patient knows no games; he dislikes the theater; he is not interested in being with other people; he does not read. He has led a very careful life, training himself with physical exercises.

The exceedingly quick tremor and the steady high pulse are very suggestive of hyperthyroidism. There is no evidence of overstimulation or of anxiety neurosis. The whole picture suggests much more an excessive grind without let-up and hobbies and the necessary lubrication in a man naturally somewhat tense and nervous. I should expect most relief from a readjustment of his life, which would permit of more adequate recreation after his probable hyperthyroidism is adjusted.

Urologist's Report.—The patient voided 4 ounces of perfectly normal looking urine. Penis normal. There is only one testicle in the scrotum, the right one, and that is perfectly normal. The left testicle can be felt as a small body in the inguinal canal; it shows marked atrophy. The left side of the scrotum is also not developed.

Anal sphincter good. The prostate is distinctly enlarged, generally smooth, elastic. The seminal vesicles are palpable, slightly distended, but soft. One gets the impression, on rectal examination, of a moderate amount of benign prostatic hypertrophy. A catheter was passed with ease and there is no residual urine.

Impression: There seems to be no indication for cystoscopy, and the patient is having no obstructive symptoms. There is present a slight grade of benign prostatic hypertrophy not producing symptoms. No indication for treatment.

Heart Station Report.—(See special electrocardiographic studies later.) As the first electrocardiographic examination revealed very interesting findings the patient was taken into the private ward at the Johns Hopkins Hospital for a period of observation and special study at the Heart Station, as it seemed worth while to analyze very carefully the peculiar cardiac arrhythmia existing.

Clinical Diagnosis.—1. Remarkable, probably unique, form of cardiac arrhythmia (to be further analyzed).

2. Graves' syndrome, including tachycardia, struma, tremor, protrusio bulborum and slight hypersensitiveness to epinephrin.

3. Chronic tonsillitis and antritis on the right side.

4. Oral sepsis.

5. Slight atherosclerosis with dilatation of the aorta, enlargement of the heart, double arcus senilis and slight nephropathy.

6. Cecal stasis, hyperacidity, and palpable liver.

7. Undescended left testicle and slight benign prostatic hypertrophy.

8. Psychoneurotic state with insomnia, depression and disturbance of the somatopsyche.

Treatment.—Feb. 20, 1918, the patient was admitted to the private ward of the Johns Hopkins Hospital for further observation and for treatment. He was kept at rest in bed and on a light diet and was given massage three times a week. His dentist treated the gingivitis. The infected antrum was irrigated. April 18 the infected tonsils and adenoids were removed by the rhinologist. April 26, 1918, he was transferred to the surgical side for the removal of a portion of his thyroid gland by Dr. Emil Goettsch. He returned to the medical service April 30, 1918, and remained for further observation and medical treatment until May 10, 1918, when he was discharged from the hospital, very much improved.

May 15, 1918, he reported that he was very much better. His own impression was that the thyroid operation had helped him more than the treatment of the teeth or the removal of the tonsils On being asked of what his improvement consisted, he said: "I feel more like I used to feel. I feel that I am getting to be all right. I can take an interest in things. I am no longer despondent. My appetite is better. I am not nervous now. Even though I don't sleep as well as I should like to sleep, I do not feel nervous. My voice is better though I am still weak. In every way I feel better." His pulse rate was 78 and regular. The fine tremor was still pronounced in the fingers. The eye signs were still present. The weight was about normal.

The patient was seen again June 24, 1918. He said that he has been getting on well; that he has had no more fluttering of his heart, though he feels an occasional dropped beat. He no longer has headaches. He eats naturally and looks and feels very well, weighing 174 dressed. He takes calisthenic exercises, walks an hour each day, and has been attending to his business, though he has been careful to avoid overexertion and mental fatigue. His pulse rate is now about 100 and is regular except for an extrasystole at about every sixtieth beat.

B. ELECTROCARDIOGRAPHIC STUDIES

DR. H. B. RICHARDSON

Electrocardiograms were taken at frequent intervals. Williams' model of the Einthoven string galvanometer made by Hindle & Co., was used. The fiber was of gilded quartz and had a resistance of 3,600 ohms. The resistance of the patient to a direct current varied from 1,500 to 3,100 ohms. In all records the tension of the string was so adjusted that a difference of potential of 1 millivolt caused a deflection of 1 centimeter. This standardization is shown graphically in Figure 4. The standardization was similarly recorded and verified in each lead in all records. The string was aperiodic in all cases. In the records, abscissae equal 0.1 millivolts, ordinates 0.04 second.

VIEWS OF VARIOUS OBSERVERS REGARDING ABNORMALITIES OF THE P-WAVE

As this study is concerned mainly with the disturbances of atrial function met with in a single patient, a brief review of the literature on the abnormalities of form of the P-wave may be of interest.

In general, according to Einthoven, Fahr and de Waart,[1] an inverted wave occurring at the same instant of the cardiac cycle in all three leads means negativity at the basal region. Specifically, an inverted P-wave in all three leads means onset of negativity at a point opposite from the normal; that is to say, low in the atrium instead of in the sino-atrial node. Lewis[2] has shown experimentally that stimulation of the atrium yields a P-wave the form of which varies according to the location of the stimulus; if near the sino-atrial node it is of normal contour; if in midatrium it tends to be iso-electric; if in the atrium it is inverted. In paroxysmal tachycardia, the P-wave is usually inverted and the stimulus is therefore held to originate low in the atrium. In atrial flutter the P-wave is almost invariably abnormal.

1. Einthoven, W., Fahr, G., and de Waart, A.: Ueber die Richtung und die manifeste Grosse der Potentialschwankungen im menschlichen Herzen über den Einfluss der Herzlage auf die Form des Elektrokardiogramms, Arch. f. d. ges. Physiol. **150**:275, 1913.

2. Lewis, T.: Galvanometric Curves Yielded by Cardiac Beats Generated in Various Areas of the Auricular Musculature. The Pacemaker of the Heart, Heart **2**:23, 3 pl., 1910-1911.

Recently Heard and Strauss[3] concluded from the study of a case that it is diphasic, primarily downward, and that "the origin of the auricular impulses is believed to be ectopic."

In atrioventricular rhythm, the P-wave is frequently, though by no means constantly, inverted. As an example of this, the observations of Williams and James[4] may be mentioned. In heart block, cases have been observed in which the P-wave that falls in the ventricular cycle is both premature and inverted (Cohn and Fraser,[5] Wilson and Robinson[6]). In *situs inversus*, of course, the P-wave is inverted in Lead I, owing to the position of the heart. Apart from these conditions, inversion of the P-wave in all three leads is exceedingly rare. Goddard[7] found among 700 records but one instance of inversion of the P-wave, and in this case the inversion was transient and confined to Lead I. In personal observation of some 400 records, we have seen it only in this case.

Hart[8] publishes, on page 73 of his monograph, a curve of Lead II in which the P-wave is inverted. The explanation of his figure reads: "Every P-wave is of an abnormal form, indicating an abnormal point of origin in the auricle, or an abnormal path through the auricular wall." No discussion is given in the text.

Apart from the conditions mentioned, then, inversion of the P-wave in all three leads is exceedingly rare, and indicates that the excitation wave originates low in the atrium.

INTREPRETATION OF RECORDS

For the sake of clearness, the records will be discussed in an arbitrary rather than in a chronological order. Those in which the ventricles beat regularly will be considered first. In Figures 1, 2 and 3, three different mechanisms are shown. In Figure 1 the rhythm is regular, the rate 112, the sequence left ventricular, and the P-R interval is 0.20 second in Lead II. The P-wave is upright in all three leads. T is primarily downward in Leads I and II, and has a suggestion of a

3. Heard, J. D., and Strauss, A. E.: Auricular Flutter. A Consideration of Some Problems Arising in the Study of a Case of the Literature, Arch. Int. Med. **20**:409, 1917.

4. Williams, H. B., and James, H.: Reversal of the Cardiac Mechanism, Heart **5**:109, 2 pl., 1913-1914.

5. Cohn, A. E., and Fraser, F. R.: The Occurrence of Auricular Contractions in a Case of Incomplete and Complete Heart Block Due to Stimuli Received from the Contracting Ventricles, Heart **5**:141, 1 pl., 1913-1914.

6. Wilson, F. N., and Robinson, G. C.: Heart Block. I. Two Cases of Complete Heart Block Showing Unusual Features, Archives Int. Med. **21**:166, 1918.

7. Goddard, C. H.: Changes in the P-Wave of the Human Electrocardiogram, Archives Int. Med. **16**:633, 1915.

8. Hart, T. S.: The Diagnosis of Abnormalities of Myocardial Function, Arch. Diagnosis **9**:31, 1916.

diphasic character. Its excursion is not over 0.25 millivolt in any lead, and it consists only of a slight, smooth undulation in Lead III. These records constituted the nearest approach of the patient to a normal rhythm. The mechanism may, therefore, be referred to as the physiologic rhythm, although distinct abnormalities are shown; that is, predominance of the left ventricle, tachycardia, a slight increase of the conduction time, and an inversion of the T-wave.

Figure 2 shows pronounced alterations. P is slightly diphasic, but chiefly upright in Lead I. It is notched in Lead II and the second portion, that which follows the nŏtch, is not so high as that which precedes it. In Lead III the notch persists and the two components of the wave are of equal height. The change in the contour of the P-waves indicates that the origin or course of the excitation wave in the atrium is different from that of the physiologic rhythm. The most striking part of the record is, however, the alteration in the form of the wave at the beginning of T. Here in all three leads is seen a small deflection, which is absent from the physiologic rhythm of Figure 1. In each lead the contour of this wave is the same as that of P. It represents, therefore, an accessory atrial contraction.

The same reasoning is applicable to Figure 3. The deflection occupying the position of T is much deeper than in Figure 1. The increase of depth may be ascribed to a combination of an accessory atrial wave and the T-wave. To produce this effect, the accessory contraction, which may be conveniently labeled P′, must be inverted. This P′ is inverted in all three leads. The same is true of P. The curve therefore shows inverted P-waves and inverted accessory atrial waves, which fall in the early portion of the T-waves. The mechanism is the same as in Figure 2, except that the waves under discussion are inverted. For the reasons indicated in discussing the literature, this inversion indicates that the excitation wave begins low in the atrium.

Against the above interpretation is the possibility that the change in form of the T-wave is due not to a superimposed wave, but to a change in the T-wave itself. The transition shown in Figure 4 tends to dispose of this objection. At the beginning, the entire curve was displaced by a potential difference of one millivolt, for the purpose of recording the sensitiveness of the string; nevertheless, the contour of the waves remains clear. The first two T-waves are of small amplitude, diphasic and primarily downward; subsequent waves show a gradually increasing, sharp downward depression. Unless the T-wave is assumed to change its shape in four beats without change in rate or modification in the contour of other waves, the depression must be due to a superimposed atrial contraction. While these P-waves are developing, the P-wave changes from a slight upward undulation to a frankly inverted wave. This curve shows a transition from a relatively

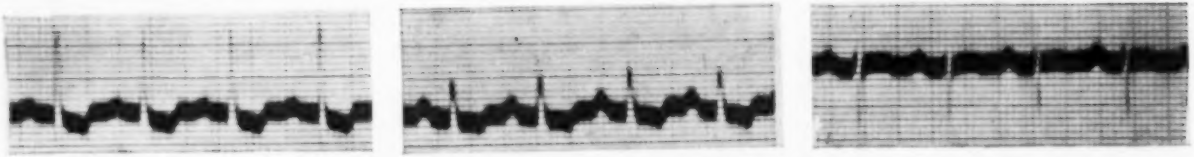

Fig. 1.—March 28, 1918. Physiologic rhythm.

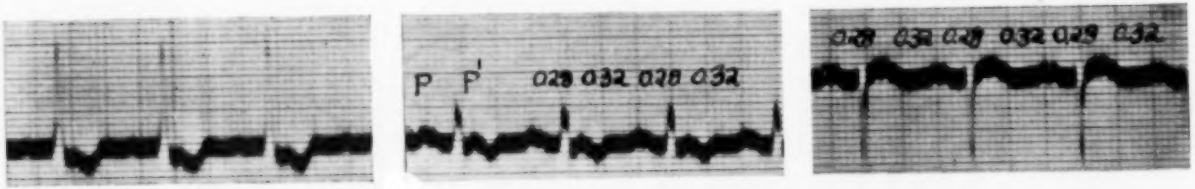

Fig. 2.—Feb. 15, 1918. P-wave notched. Blocked alternate atrial extra-systoles.

Fig. 3.—March 16, 1918. P-wave inverted. Blocked alternate atrial extra-systoles.

normal rhythm to one in which the excitation wave begins at a point low in the atrium, and in which an accessory contraction of the atrium occurs during systole of the ventricle.

Three possibilities suggest themselves as explanation for the appearance of this wave. The first of these is atrial flutter with a 2:1 atrioventricular block. In flutter, as ordinarily understood, the atrial waves are regular. A glance at Figures 2 and 3 is sufficient to show that this is not the case in the present instance. The accessory atrial contraction occurs prematurely. If it be designated as P′, then in Leads II and III of Figure 2, in which the waves offer the most definite points for measurement, the P-P′ interval measures 0.28 second, whereas, the P -P interval measures 0.32 second. These figures are identical in each of five cycles. In none of the electrocardiograms of the eleven cases of flutter on file in our records is there an instance of such an arrhythmia, nor have we encountered it in any of the published electrocardiograms of flutter. A tracing obtained from this

Fig. 4.—March 6, 1918. Showing transition from physiologic rhythm to alternate blocked atrial extrasystoles.

patient at another time demonstrated atrial flutter and differed radically from the one under discussion (Fig. 7). Atrial flutter, as ordinarily understood, may therefore be excluded.

Another possibility is the retrograde stimulus of the atrium by the contracting ventricle, either by a retrograde excitation wave or by a mechanical stimulus. The former mechanism[4] has been recorded in atrioventricular rhythm, the latter in complete heart-block,[5, 6] that is to say, in rhythms in which the ventricle is the pacemaker. Neither has been observed, as far as we are aware, in the course of a sequential rhythm. A third possibility is that the P′-waves are due to atrial extrasystoles that evoke no ventricular response. They occur so early that a fatigue block is readily conceivable, especially in view of the slightly prolonged P-R interval of 0.20 second. Additional tracings were required to decide between the last two alternatives.

An example of these is shown in Figure 5. In Lead II the first two cycles indicate the same mechanism as Figure 3; that is, an abnormal path of the excitation wave in the atrium and accessory atrial

contractions. The third ventricular complex is premature. It follows 0.28 second after P′ without an intervening P. Evidently this ventricular complex is a response to the atrial contraction P′, the diminished conductivity being explained by the fatigue caused by the short diastole. This P′ becomes P also, and the expected P-wave falls in the ventricular cycle, thus becoming what has been called an accessory atrial contraction. After this change of step the mechanism proceeds as before. Three similar ventricular responses are shown in Lead I and one in Lead III; in these instances the P′-R interval is 0.24 second, which is not much greater than normal. The accessory atrial waves that fall in ventricular systole are thus capable themselves of eliciting

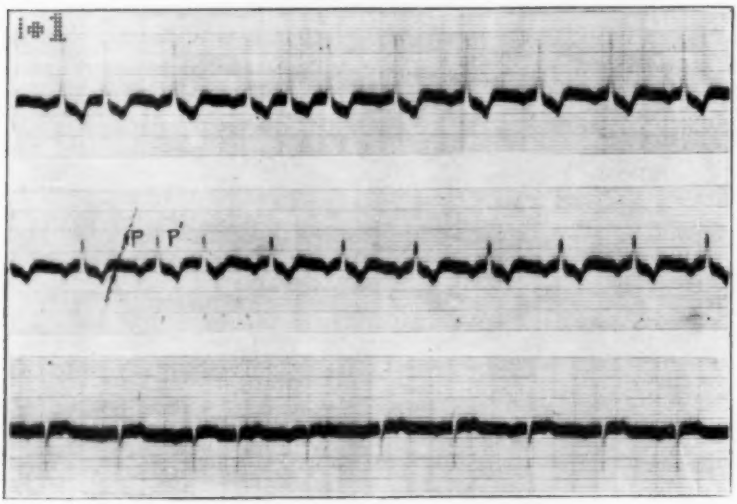

Fig. 5.—March 6, 1918. Inverted P-wave. Alternate atrial extrasystoles, usually blocked, sometimes not blocked.

a ventricular response. Thus the mechanism is alternate atrial extrasystoles of the same origin or path as the sequential atrial waves, or what might be termed an atrial pulsus bigeminus. These extrasystoles are usually blocked but often provoke a ventricular response, though after an interval that is somewhat greater than normal. Attempts to explain the mechanism on the basis of a stimulus passing backward from ventricle to atrium become very involved.

In Figure 6 is shown essentially the same mechanism, with the exception that more of the atrial waves are followed by a ventricular response. When this occurs several times in succession it constitutes a short paroxysm of tachycardia in which the rhythm is regular. The

figure begins with four such beats; then comes a pause, a cycle of the rhythm of Figure 3, and then a relatively normal P-wave followed by a paroxysm of seven rapid beats. The R-R intervals indicated on the figure show that the rapid rhythm is regular except for the last interval, which is a trifle shorter. The portion of the curve that lies between the R-waves varies in contour, but the P-waves are discernible. After this short run of tachycardia the mechanism of blocked alternate atrial extrasystoles is resumed.

Still another mechanism is shown in Figure 7. It is perhaps well to recall that during the physiologic rhythm of Figure 1 the T-wave in Lead II consisted only of slight undulation. In Lead III of Figure 7 there is a series of regular waves between each two ventricular beats. These waves are seen to be continuous throughout the systole and diastole, though they are modified in shape when mixed with the ventricular complexes. The curve is a definite picture of atrial flutter at an atrial rate of 300. The ventricle responds usually to every fourth, occasionally to every third, atrial contraction. In Lead I the same mechanism is in evidence, though it is less characteristic. In Lead II, however, the mechanism is not the same. The record differs in no

Fig. 6.—March 14, 1918. Two short paroxysms of tachycardia with regular rhythm. Reversion to blocked alternate atrial extrasystoles.

essential from that in Figure 3. Two inverted P-waves and two only are distinguishable in the cardiac cycle, one in the normal position and one, definitely premature, fused with the T-wave. Although both these waves are pronounced, no intermediate atrial waves can be seen. The mechanism is evidently different from that of the other leads and consists of inverted P-waves and blocked alternate atrial extrasystoles. As the leads were taken in chronological order, the change of mechanism during the shift from Lead I to Lead II and back again during the shift from Lead II to Lead III is something of a coincidence, yet the records seem to permit of no other interpretation.

OBSERVATIONS ON COMPRESSION OF THE VAGUS

The vagus was stimulated by digital compression of either or both nerves over the carotid artery in the neck. Continuous electrocardiograms were taken, including the period of compression and a few beats before and after. The rate before vagus pressure was compared with that during the four or five beats that included the release of pressure.

Results varied according to the mechanism present. The effect on the physiologic rhythm was recorded on four occasions, as follows:

	ATRIAL RATE	
	Before	At Release
Right vagus	83	64
Right vagus	111	104
Left vagus	82	71
Left vagus	107	104
Both vagi	107	96
Both vagi	112	107
Average	100.4	91.0

Thus marked slowing resulted.

On the mechanism of blocked alternate atrial extrasystoles, with P-wave upright but abnormal (Fig. 2), one observation was made.

ATRIAL RATE	
Before	96
At Release	96

Here no change resulted.

On the mechanism that resembled the preceding except that the P-wave was inverted and that not all the extrasystoles were blocked (Figs. 3, 5 and 6), observations were made on three occasions, as follows:

	ATRIAL RATE	
	Before	At Release
Right vagus	141	140
Right vagus	103	105
Left vagus	140	138
Left vagus	165	168
Both vagi	160	163
Average	141.8	142.8

Here no change resulted.

On atrial flutter one observation was made.

	ATRIAL RATE	
	Before	At Release
Right vagus	300	300

Thus no change resulted.

The average of all observations on subnormal atrial mechanism was:

ATRIAL RATE	
Before	157.9
On Release	158.6

In no instance did vagus pressure produce any change of mechanism except that it tended to prevent ventricular response to atrial extrasystoles. Examples of vagus pressure on physiologic and abnormal mechanisms are shown in Figure 8.

Thus, pressure on the vagus nerves caused a marked reduction in atrial rate during the physiologic rhythm and no change during the activity of an abnormal atrial mechanism. The observations have been described in detail because they have a bearing on the latter. The inversion of atrial waves might be ascribed either to a dislocation of the pacemaker or to an abnormal path of the excitation wave in the atrium analogous to that which occurs in the ventricle when there is a defect in the Purkinje system. If the latter alternative be correct, and the impulse arise in the sinus node, it is to be expected that pressure on the vagus would reduce the atrial rate to the same degree as occurs during the physiologic rhythm. That this does not take place is evi-

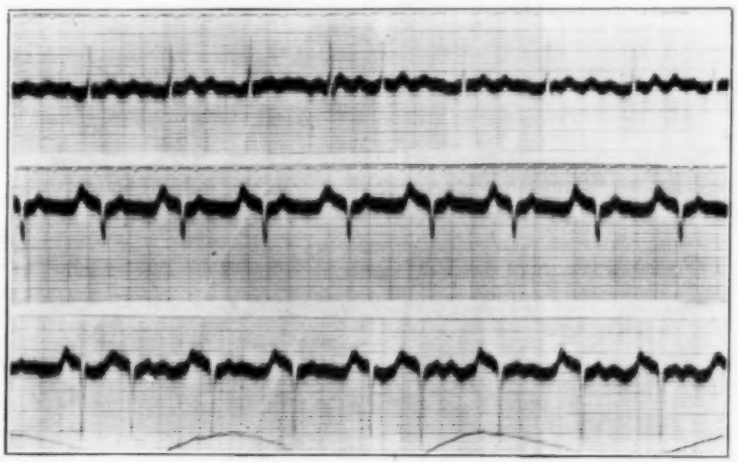

Fig. 7.—March 4, 1918. Atrial flutter. Inverted P- wave. Blocked alternate atrial extrasystoles.

dence that in the abnormal rhythm the pacemaker is dislocated. This case would seem to offer no support for the view that there is in the atrium, as in the ventricle, a specialized tissue subject to damage, which conducts the excitation wave, as concluded by Eyster and Meek,[9] and recently contested by Lewis.[10]

The failure of pressure on the vagus to produce any radical change of mechanism is evidence that in causing the dislocation of the pacemaker the vagus plays no part.

9. Eyster, J. A. E.: Experiments on the Origin and Propagation of the Impulse in the Heart, Heart **5**:119, 137, 3 pl., 1913-1914.

10. Lewis, T., White, P. D., and Meakins, J.: The Susceptible Region in A-V Conduction, Heart **5**:289, 1913-1914.

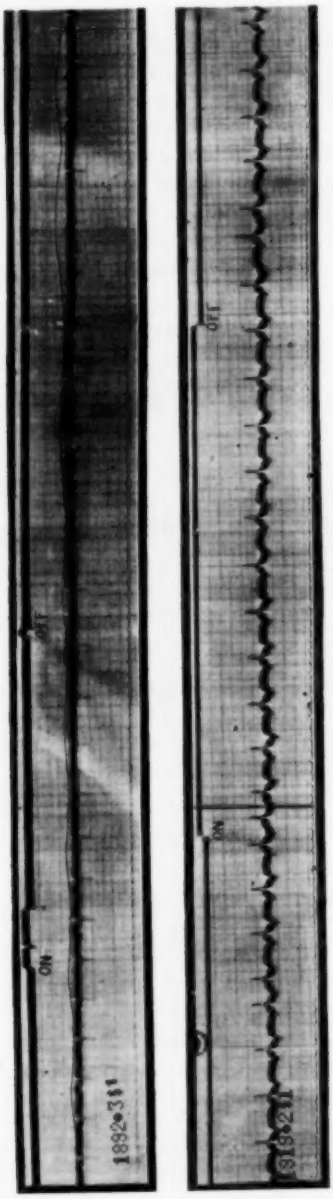

Fig. 8.—Upper curve, Lead III, Feb. 25, 1918. Effect of pressure on right vagus on physiologic rhythm. Lower curve, Lead II, March 11, 1918. Effect of pressure on right vagus on abnormal rhythm.

OBSERVATION ON EFFECT OF ATROPIN

One observation was made on the effect of atropin; $\frac{1}{60}$ grain of the sulphate was injected subcutaneously. Curves taken five minutes before showed the physiologic rhythm and a slowing of ventricular rate by pressure on both vagi from 105 to 99. Curves were taken at 5-minute intervals for 45 minutes. The effect of atropin was demonstrated by the symptom of dryness of the mouth and by the fact that vagus pressure one-half hour afterward had no effect on the rate. Although the drug had thus a well marked effect and increased the rate from 105 to 130, it produced no change in the mechanism. Depression of the vagi by atropin, like stimulation of them by pressure, failed to produce a change of mechanism. No evidence was obtained that the abnormal mechanism is related to vagus tone.

SUMMARY OF ELECTROCARDIOGRAPHIC STUDY

If the above interpretations are correct, we have observed in this patient at one time or another the following:

1. Physiological rhythm.
2. Dislocation of the pacemaker from the sino-atrial node to points elsewhere in the atrium.
3. Alternate atrial extra-systoles, none of which provokes a ventricular response.
4. Alternate atrial extrasystoles, many of which provoke a ventricular response.
5. Paroxysmal tachycardia.
6. Atrial flutter.

It is hoped that the very unusual combination of atrial disturbances presented by this case may be welcomed by those who desire to throw light on abnormal functioning of the atrium of the heart.

PITUITARY HEADACHES AND THEIR CURE*

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Headache as a symptom is well known to all. The patient who complains of it is too often given a cathartic and aspirin and dismissed without adequate study to determine its true etiology. He may then go elsewhere and return after he has developed more definite signs. I remember one patient who returned four days later with the complete picture of a tuberculous meningitis.

Headache commonly presents itself to us as the symptom of some systemic disease, such as circulatory disturbance, especially hypertension, nephritis, anemia, an acute febrile disease, or poisoning by various toxic substances. These may be exogenous or endogenous; for example, lead, carbon monoxid, alcohol, or the toxins resulting from acidosis, diabetes, uremia, or from the gastro-intestinal tract. Syphilis is a frequent cause of headache. There are likewise local causes in disturbances of the cerebral circulation, a myositis of the occipitofrontalis muscle, periostitis or gumma of the cranium, inflammation of the accessory sinuses, increased intracranial tension resulting from abscess, meningitis, tumors, etc., errors of refraction and eyestrain, or migrain which may prove to be an intermittent claudication of the cerebral arteries. Certain headaches are of reflex origin, as those which come from menstrual irregularities or pelvic disease. Headache may be a symptom of hysteria and the various neuroses and psychoses. So much for the multitudinous causes to which we often ascribe this distressing symptom.

The part which the pituitary gland may play in the production of headache is frequently overlooked. The clinical picture of severe frontal headache, somnolence, mental dulness, polyuria, increased sugar tolerance or glycosuria, oculomotor palsies, bitemporal hemianopsia, and evidence of disturbed bony metabolism or adiposity, sexual regression, etc., is a well known syndrome caused by pituitary tumors. But it is the early and less easily recognized pituitary disturbances on which interest must center, for that is the stage in which therapy will help; and one of the first symptoms of a pituitary gland which is functionating improperly is a frontal headache which does not yield to the usual remedies.

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* From the Third Division of the Neurological Institute.

It is this type of headache which I wish to present in all its phases, hoping that it may aid a little toward the knowledge of the therapeutic value of pituitary. Several case reports added at the end will show the clinical value of these observations.

ETIOLOGY

The cause of pituitary headaches is, briefly, an enlargement of the hypophysis following on a stimulus or demand made on it, or a pathologic condition of the gland. It is more common in women than in men, and may occur at any age, though it is most frequently seen in adolescence and early adult life.

PATHOLOGY

Among glandular structures the anatomy of the pituitary gland is unique, in that it is situated at the base of the brain, connected to it by a narrow stalk, the infundibular process, and lying within the confines of the sella turcica, a bony crib to whose physical limitations it is subject. The size of the hypophysis is quoted by various authors,[1] averaging in the sagittal plane from 6 to 10 mm., vertical from 10 to 14.5 mm., transverse from 5 to 9.75 mm., the weight 0.6 gm., comparing favorably in size to the hazel nut. The gland, made up of three parts, is surrounded by a thin capsule—a continuation of the dura mater; anterior to it lie the olivary eminence and the anterior clinoid processes; below, the dorsum sellae; behind, the posterior clinoid processes; and above, overhanging the entrance to the sella in the form of a diaphragm, is a firm prolongation of the dura which is perforated by the infundibular process. The nerve supply is derived from sympathetic plexuses along the carotid artery. Tilney,[2] in an admirable article, has contributed largely to the phylogenesis and anatomy of the hypophysis. To Cushing[3] and Falta[4] we owe important contributions to its physiology and clinical significance.

From the foregoing it can be seen that the pituitary gland is entirely surrounded by a firm framework, on three sides by bone, above by dura and only on the lateral aspect is there any opportunity for expansion. If the gland were situated in masses of loose areola tissue such as the thyroid or the adrenals, a considerable enlargement would be possible before pressure symptoms were felt or could even be detected; but, let the hypophysis enlarge just 2 or 3 mm., and there will develop

1. Zander: Quoted by Munson and Shaw, Archives Int. Med. **14**:493, 1894. Hitchcock: Med. Rec., New York, Sept. 10, 1911.
2. Tilney: Pituitary gland. Mem. Wistar Inst., 1911.
3. Cushing, H.: Pituitary Gland and Its Disorders, 1912.
4. Falta: Ductless Glandular Diseases.

a train of symptoms dependent on this mechanical discrepancy. We see, then, that there are two factors which enter into the production of these headaches, the size of the sella turcica and the size and pathologic condition of the pituitary gland.

In a series of twelve cases showing no dyspituitary signs or symptoms, normal sella turcica measurements are shown in Table 1.

TABLE 1.—Normal Sella Measurements as Determined by the Author

Name	Antero-posterior	Depth	Name	Antero-posterior	Depth
A	11	10	F	10	8
D	11	10	S	11	7
B	12	10	M	11	9
M	10	8	E	11	10
P	10	8	H	11	8
A	9	8	M	11	10
Average	9–12	7–10			

Average measurements quoted by other writers are as shown in Table 2.

TABLE 2.—Measurements of Sella Noted by Various Observers

Name	Anteroposterior	Depth
Keith[5]	10–12	8
Potts[6]	8–13	6–10
Fearsides[7]	10–12	8

To compare with the foregoing a series of fifteen cases which showed distinct dyspituitary signs, Table 3 reveals either an enlargement of the sella or else a definite contraction:

TABLE 3.—Comparison of Cases Showing Dyspituitary Signs

Name	Antero-posterior	Depth	Name	Antero-posterior	Depth
D. S.	12	9	R. W.	17	13
E. H.	11	11	S. F.	9	8
J. T.*	15	11	E. M.†	30	30
E. F.	14	12	D. S.*	12	8
S. J.	11	8	W. G.*	9	7
E. W.*	12	12	N. C.*	15	13
S. W.	12	10	N. F.*	15	8
J. L.	13	12	M. B.*	5	5

* Cases marked with * are measurements of sellae of case reports.
† E. M. is a case of pituitary tumor with acromegaly.

Another table will serve to show the comparative measurements of the normal hypophysis and the sella turcica (Table 4):

TABLE 4.—Comparative Measurements of Normal Hypophysis and Sella

	Sella-Turcica	Hypophysis
Anteroposterior	9–12	6–10
Vertical	10–14	7–10

5. Keith: Lancet, London, 1911, **1**, 993.
6. Potts: Jour. Am. Med. Assn., 1913, **61**, 1188.
7. Fearsides: Lancet, London, 1914, **2**, 16.

From these figures and facts we determine that normal pituitary glands usually have normal sella conformation, but should the glandular function be disturbed there will result certain signs and symptoms and the roentgenogram will show an abnormal sella turcica.

Roentgenograms of this type of case having "pituitary" headaches and dyspituitary signs reveal a wide variety of sellae turcicae and I should like to note here the importance of a careful study of each plate by the clinician with a standard normal plate as reference. There may be a very small contracted fossa with clinoids in apposition, or there may be a large fossa with a similar formation of clinoid processes; the former of these would be expected to show hypopituitary and the

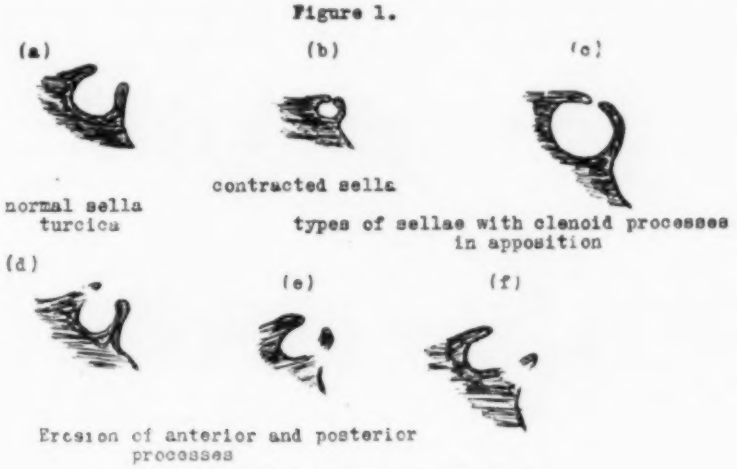

Fig. 1.—Various types of sella turcica.

latter hyperpituitary signs. Then again, there might be evidence of erosion of the posterior clinoid processes with, perhaps, pyramidal tract signs, or of the anterior processes with bitemporal hemianopsia or contraction of the visual fields, indicating in the former, enlargement of the gland posteriorly and in the latter anteriorly. There is also the generally enlarged sella turcica, the sella whose posterior process is tilted backward; and last of all, the complete destruction of the fossa and dorsum sellae, such as occurs in neoplastic overgrowth of that region. If a careful study of the physical signs and symptoms of the patient is made prior to the roentgen ray picture, the conformation of the fossa can frequently be prognosticated with considerable accuracy. Drawings of various types of sellae are shown in the accompanying diagram (Fig. 1).

Why the pituitary gland should enlarge is the next question that comes to mind. It is well known that during menstruation and pregnancy there is a physiologic enlargement of the gland, Falta noting an increase in weight from 0.6 to 1.0 gm. This enlargement may be cyclical and account for the periodical occurrence of the menses, or else we can ascribe it to stimulation from the gonadal secretion. Other glandular disturbances are also liable to influence the pituitary, etc., such as pineal or thymic subinvolution, adrenal or thyroid disease, which is most probably in the nature of a compensatory phenomenon.[8] A mental shock or traumatism to the skull may be the exciting cause to an altered hypophyseal function. These occasionally result in a hyperplasia of the glandular elements, but the gland may be also the seat of colloid degeneration, cysts, abscess, hematoma, adenoma, adenocarcinoma, sarcoma, endothelioma and teratoma.

In many ways, therefore, we see there can occur a disproportion between the pituitary body and the sella with the production of pressure on the sensory nerves to the dura; and by its encroachment on the cavernous sinuses it may cause interference with the cerebral circulation, the whole setting up the train of pituitary symptoms, including the headache.

SYMPTOMS AND SIGNS

The pituitary headache has three characteristics: its location; its duration and persistence; and its relief under specific medication. A patient will come to the physician complaining of a frontal headache. On questioning further he will say that it is situated "deep in the forehead behind the eyes," often feeling as though it were pressing on them, giving a "dazed" sensation. Not infrequently, without asking, and always on asking, the patient, placing the finger on either temple, pointing directly inward at the hypophysis in the attitude of the accompanying picture will say, "Doctor, it is between here" (Fig. 2). Depending on its severity, it is described as a tightness between the temples, a feeling of pressure or distention, or an intense, bursting ache. Rarely they complain that there is a sensation of "something in there" and on moving the head, they may feel as though "a marble-like object were rolling about." Deep pressure on the temples may elicit some tenderness.

This headache is very persistent, usually lasting from one-half hour to forty-eight hours, and it may be continuous, frequently coming on in the female at the time of the menses. It often leaves very suddenly, returning again with exacerbations; it is accentuated by excitement, stooping over, and by the ingestion of sugar. At the climax of the headache we may see nausea and vomiting, with which there will come

8. Timme, W.: New York M. J., Oct. 16, 1915.

relief. Marked fatigue accompanies the headache, the patient hardly being able to drag himself about, and there is present to stroking a broad white skin line as evidence of suprarenal deficiency caused by the drain on the adrenal function by the exhausted pituitary. The patients feel slowed down in their activity, yawn excessively, are sluggish and willing at any moment to seek an opportunity for sleep. These patients are particularly prone to attacks of depression, which come on without any cause, and have as their basis some very insignificant fact. In children there is apt to be evidence of mental retardation, with dulness, sluggishness of the mind and lack of the higher reasoning powers, this usually occurring in hypopituitary conditions, while in adults we sometimes see a loss of moral control resulting in frequent visits to the police courts. The menstruation has certain characteristics in these pituitary individuals. It may begin very early, at the age of

Fig. 2.—Location of pituitary headache (Case 7). Note the coarseness of features and the nasal eyebrow.

10 or 12, or else very late, at 16 or 18. The periods are irregular, often coming every two or three weeks, and the flow excessive. Sexual development may be very precocious in the hyperpituitarics. Polyuria is occasionally present and constipation frequently accompanies the height of the headache with diarrhea at its termination.

Knowing that the pituitary, together with the adrenals, controls the mobilization of sugar in the body, it is not strange that these patients should have anomaly of sugar metabolism, as is seen in the periodic development of an intense craving for sweets, a sort of dipsomania, as it were, for sugar. The satisfaction of this desire being completed by eating candy, it is almost invariably followed by a typical pituitary headache. We can readily see that owing to the increased demand on it, there is an enlargement of the pituitary gland, and following on this the adrenals are called on to assist in mobilizing the sugar, the excessive drain on them causing great fatigue and the formation of a vicious circle.

How, then, are we to recognize these cases and tally their physical signs with the symptoms enumerated? Do we expect to see finished acromegalics or giants, or adiposis with sex regression? No! but there are certain dyspituitary signs which are of aid in diagnosis.

The growth of hair has certain peculiarities; it is more apt to be dark and coarse in texture. The amount is abundant, the arms, legs and body being covered with a quantity of hair; the pubic hair not infrequently is of masculine type in the female, growing up to the umbilicus in a triangular fashion and in the male it may be of the female type, straight across over the mons veneris. The eyebrows are heavy and long and they often meet in the midline over the nose forming a nasal eyebrow. The female may show a tendency to a mustache. The bony framework is altered, there being either excessive length or breadth of bone, depending on whether or not the epiphysis had united when the metabolism was disturbed. The appearance of the face might show one or all of the following: eyes too close together or too far apart, a large nose, prominent superior maxillae, a prognathism of the lower jaw, a general coarseness of the features with thickening of the lips. The teeth, especially those of the upper jaw, either widely spaced or else unusually broad. These persons are sometimes very tall, with large hands and feet, and there is a broadening of the hands, with clubbed fingers. Deposition of adipose tissue may be excessive. Pulse and blood pressure are both apt to be low, especially during the headache, this being more an expression of adrenal exhaustion than pituitary. Blood sugar determination and sugar tolerance will in most of these cases reveal either too high or too low a figure. A contraction of the temporal fields of vision is occasionally found and, rarely, a primary optic atrophy.

TREATMENT

Specific remedies for the cure of disease constitute the treatment *par excellence* and modern medicine has given to us a number of such agents; for example, arsphenamin for syphilis, the employment of serums in pneumonia and epidemic meningitis, thyroid in myxedema and cretinism, etc. Such a specific is the administration of pituitary to cure pituitary headaches.

There are a number of good pituitary preparations on the market, the most satisfactory being Armour & Company's and Burroughs Wellcome's tablets of the whole gland. If the latter are used they should be prescribed in doses four times as large as any others. We commonly use Armour's tablets in doses varying from ¼ grain to 2 grains three times a day; an average for an adult is 1 grain, preferably given one hour after meals. Much larger doses are recommended by some authors, Cushing giving as much as 15 grains in some

of his cases. For a more rapid action it is claimed that hypodermic injection of pituitary extract, 0.5 to 1 c.c., is valuable, but I have seen better results with preparations of the whole gland.

Continuous medication with pituitary will result within a few days in a decrease in the intensity of the headaches; there will be a longer period between their occurrence, the head will feel less "tight," and fatigue, nausea, and vomiting will also disappear. An examination of the case reports given later will show very gratifying results. A number of these cases cure themselves, and it is the belief of Timme[9] that the person who has a small sella turcica with dyspituitary signs and symptoms will remain so indefinitely, even though pituitary administration helps toward relief; but if the sella is large and there is room for expansion, there will occur the foregoing type of symptoms and the patients will eventually cure themselves, even though they become acromegalic in the process. We can therefore regard symptomless acromegalics as a finished product, according to this investigator.

But there are also those cases which, having symptoms of pituitary disease, do not improve on treatment, and it is then that we must begin to consider the possibility of a neoplasm and careful observation becomes essential. Just a word of caution against too long continued pituitary therapy. After the symptoms show improvement, diminish the dose and give it only three or five days out of the week; this gives the gland a chance to readjust itself.

Case reports follow, and as they are all from clinic patients, it has, unfortunately, been impossible to have blood sugar and sugar tolerance tests made.

CONCLUSIONS

1. Pituitary disturbances constitute a fairly common cause of headache.

2. Pituitary headache is located between the temples, deep in behind the eyes and is accompanied by dyspituitary signs.

3. Abnormality of the sella turcica is demonstrable in almost every case of pituitary disease.

4. Administration of the whole gland cures these headaches and the accompanying symptoms in a large percentage of cases, provided there is not a progressive neoplastic growth.

REPORT OF CASES

CASE 1.—J. T., a woman, aged 40, married, had severe frontal headaches beginning at the age of 15, with the onset of the menses. The headaches were infrequent, occurring usually once a month at the menstrual periods until six years prior to observation, since which time they have increased in severity and frequency so that they now come on several times a week. The head-

9. Timme: A New "Polyglandular Syndrome," "Endocrinology." **2**:209, 1918.

ache lies between the temples and moving the head accentuates it. Vomiting has occurred about once in two weeks lately. The patient is fatigued at times and has polyuria, and the menses are always irregular. There has been no loss of appetite or craving for sweets. At the age of 30 she suddenly became very obese and increased in weight to 185 pounds. An aunt has goiter.

Physical examination shows a well developed woman, short, obese, with small hands and feet. The head is large, eyes set close together, jaw prognathous. The eyebrows are heavy, with nasal eyebrow marked. The teeth are small and not crowded, and fingers are broad, short and clubbed. The roentgen ray shows a general enlargement of the pituitary fossa, with slight erosion of the posterior clinoid processes (see Table 3, J. T.).

Pituitary, ¼ grain twice a day, was given. In one month the headaches and other symptoms had greatly improved. At the end of four months, during which time the dosage was increased to 1 grain daily, she said that she had only had two very slight headaches in the previous two months, and had had no nausea or vomiting for three months. Discontinuance of the drug caused a return of the old symptoms. This is evidently a case which originally began as hypopituitary and is now endeavoring to compensate and enlarge.

Case 2.—D. S., aged 16, schoolboy, was brought to the hospital with a history of several blows on the head, complaining of momentary "staring" spells, followed by a very severe headache, with which he would go to bed. This headache is made worse by playing or excitement and is in the pituitary location. He has stood very low in school, is sleepy, dull and stupid. When younger he had enuresis and has always been fond of candy. Mental examination showed him to be a high grade imbecile, measuring 7 years on the Terman score, not being oriented as to time, and lacking in reasoning ability and judgment.

Physical examination showed a small boy who appears about 10 years of age; structural and sexual growth are backward, there being no secondary sex signs as yet. He has rather a square jaw, teeth are broad but not spaced, the eyebrows are normal in size and the hair growth is scanty. A roentgenogram of the skull shows a slightly enlarged sella turcica with heavy clinoid processes which completely roof over the fossa. (Table 3, D. S.)

On the evidence of the headaches, the roentgenogram, and a hyperpituitary type of father, the boy was given pituitary, ½ grain three times a day. He returned in two weeks without headaches. At the end of a month the headache seemed gone for good, and there was as well a cessation of the "staring" spells. The father noted joyfully that he was less mischievous and more active. The dose was increased to 1 grain three times a day. After three months the boy says that he feels as though a "weight had been lifted off his mind," an observation he could not have made at the onset of his treatment. He is brighter, more interested, and is anxious to learn. He can tell date, year and season, and is standing much higher in his studies. Another mental test showed an advance of one year in three months' time. This is evidently a hypopituitary case which without treatment would never have come out of the imbecile class.

Case 3.—E. W., woman, aged 24, single, came into the clinic suffering with severe frontal headache, slunk into a chair holding her head, yawned frequently, extremely fatigued, dull, drowsy, nauseated, trying to vomit. She had been this way for three days. As long as she can remember she has off and on had these terrible headaches. Since coming to America four years ago they have increased in frequency and severity; she is very irritable, is constipated, and has lost 42 pounds in weight. Her headaches are paroxysmal in type, lasting for a period of from a few hours to three days; are situated between the temples, and bore in like a knife, causing an aching of the eyes at times; and with the onset of vomiting they are often relieved. There has been no menstrual irregularity. The patient has at times an intense craving for candy. Her father's sister had similar headaches.

The patient is of medium height, large frame, skull is also large, eyes are far apart, jaw slightly prognathous, teeth broad and spaced, eyebrows heavy, with a marked nasal eyebrow; the feet are small. There is a slight but definite contraction of the temporal fields of vision. The fundi show the veins engorged. The roentgenogram shows a rather large sella with clinoid processes in apposition and beginning erosion anteriorly. (Table 3, E. W.)

Pituitary, 1 grain three times a day, was given; increased after three weeks to 2 grains three times a day. In a week there was an improvement in the intensity of the symptoms and in a month the headache had diminished so that the patient has only had a slight ache once in two weeks. There has been no nausea or vomiting, fatigue is very much less, and she is anxious to return to work. She seems much brighter and more active mentally; there is less visual field contraction, and she has gained 5 pounds in weight. Improvement is continuing.

Case 4.—N. C., a nurse, aged 29, single, when a little girl had headaches all the time, which were made light of by her family because of her robust physique. She was given glasses, which have been frequently renewed, all with no relief. Headaches are severe, coming on in exacerbations in the "pituitary" location, bursting in type and accompanied until one year prior to observation by nausea and vomiting and confining her to bed. Although a large, strongly built woman, she is always fatigued and sleepy and when the headache is most intense she is dull, drowsy and wants to sleep. Several years ago she noticed that on satisfying her craving for sugar the headache became worse, so she does not eat it now. She is constipated at times and has polyuria; also has mild periods of depression. The menses began at 12 years and have been irregular ever since.

The patient has always been big, coming from a tall family. The hands and feet are large and the maxillae are prominent. The eyebrows are bushy and continuous across the nose. The hair growth is plentiful and there is a slight tendency to the masculine pubic hair. The roentgenogram shows a generally enlarged sella turcica, wide open and very deep. (Table 3, N. C.)

Pituitary, 1 grain twice a day, was given, followed by a cessation of the headaches and a diminution of the fatigue within twenty-four hours. The patient since has had only one slight recurrence of the headache, and fatigue and dulness are much improved.

Case 5.—N. F., a nurse, aged 27, single, had her first headache at 16, which was very severe, and at intervals ever since they have recurred. Coal tar drugs were often taken, with only slight relief. The menses began at 14, then skipped six months, and became regularly established at 16, since when they have been irregular. The headache usually comes a week before the catamenia and continues until the onset; then relief comes. It is typically pituitary in location, between the temples and on top of the head, described as a feeling of tightness, pressure and distention, accompanied by fatigue and occasionally depression. The patient faints easily, has excessive perspiration of the hands and cyanosis of the extremities, and has also a desire for sweets, satisfaction of which causes headache.

The patient is small, thin, square built; skull round, jaw broad, prognathous; hands and feet small, short, stubby; eyebrows heavy, with marked nasal eyebrow; tendency to moustache. Hair growth is excessive on hands and legs; pubic hair masculine in type. A roentgenogram shows that the sella is enlarged anteroposteriorly and there is slight erosion of the posterior clinoid process. (Table 3, N. F.)

Pituitary, ½ grain three times a day. In three days the patient noticed that the head did not feel quite so "tight" between the temples. The next period was preceded by a headache, which was not so severe, and the second was accompanied by only a slight dazed feeling. The headache is now gone.

Case 6.—M. B., a cook, aged 31, married, five years prior to observation had very severe headaches previous to the birth of her first child; since then

she has suffered with them off and on, becoming worse during the previous six months, lasting a whole day, forcing her to bed, and obtaining relief only by vomiting. She had glasses fitted and teeth extracted without relief. The headaches are described as severe, situated between the temples, behind the eyes, accompanied by fatigue, dizziness and sleepiness. She feels "dopy," sluggish and has no ambition, becomes depressed and unhappy and does not wish to be bothered. The menstruation began at 14 and comes every three weeks. She has no desire for sweets.

The patient is a large, obese woman, weighing 164 pounds, with scanty growth of hair. The teeth are broad, eyebrows heavy, with only a few hairs over the nose; the jaw is broad and slightly prognathous. This woman is hypopituitary in most features and the roentgenogram was no surprise when it revealed an extremely small, contracted sella turcica, with heavy clinoid processes meeting in the midline. (Table 3, M. B.)

This patient has been under treatment only a few months, but in that time has had no suspicion of a headache and says voluntarily that she feels less tired and dopy and is brighter and more ambitious. Medication has been 3 grains daily of the whole gland.

Case 7.—W. G., a sailorboy, aged 23, single, has had severe frontal headache for five years. The tonsils were removed and glasses procured without relief. The headache is boring, bursting in type, deep in the midline between the temples, as in his picture (Fig. 2). It is accompanied by extreme fatigue and at times the patient has a craving for candy. He is depressed frequently, feels mentally sluggish, his grasp is slow and his memory not so acute as formerly. He says that his hands and feet are larger and his face broader.

The patient is of medium height, has coarse, heavy eyebrows, with nasal accentuation, his nose and lips are large, features coarse, jaw prognathous, lower teeth spaced, the upper broad, hair growth is abundant, masculine type, and the hands and feet are large and square. The roentgenogram shows a small, flat sella with beginning erosion of the anterior clinoid processes. This is a hyperpituitary case which approaches more the acromegalic than any of the others.

Pituitary, 1 grain three times a day, was given. In one week the head felt clearer than in a number of years and the ache was less marked. The patient now has only a very slight headache at times, but notices particularly that his mind is more active, more attentive, and that he has a better grasp.

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A STUDY ON THE ETIOLOGY OF CHOLECYSTITIS AND ITS PRODUCTION BY THE INJECTION OF STREPTOCOCCI *

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In 1914, Rosenow,[1] by making cultures from the emulsified wall of the gallbladder of selected patients, found streptococci in most instances, and reproduced cholecystitis in animals by injecting intravenously the freshly isolated organisms. The work recorded in this report is similar to that of Rosenow, except that all gallbladders removed in operations in the Mayo Clinic, regardless of the degree of pathologic changes, were cultured.

The tissues were cultured as soon as possible after their removal, every effort being made to prevent contamination. Immediately before emulsifying, the tissues were thoroughly washed in large volumes of physiologic sodium chlorid solution. They were then ground in mortars within sterile air chambers or in a hood, the air of which was washed by means of steam from a sterilizer fastened to the end of the hood. The operator wore gloves and sleeves which, with the materials used, were sterilized in the sterilizers opening into the hood.

The emulsions thus made were inoculated in varying concentrations into tall columns of dextrose brain broth, blood broth, litmus milk, ascites dextrose broth, ascites dextrose agar and dextrose agar. Krumwiede plates of dextrose blood agar and plain blood agar plates were poured also. The cultures were studied at the end of twenty-four hours, but those that were negative were examined daily for a week.

Altogether, cultures were made from seventy gallbladders and four ulcers. At first cultures were also made from the contents of the gallbladders, but because of the large number of negative results, regardless of the findings in tissues, this was abandoned.

The duration of the symptoms in the cases studied ranged from three months to thirty years. The pathologic changes ranged from slight to marked thickening of the walls. The results of the cultures are summarized in Table 1.

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* From the Division of Experimental Bacteriology, Mayo Foundation.

1. Rosenow, E. C.: The Newer Bacteriology as Determined by Special Methods, J. A. M. A. **58**:903, 1914; ibid., Etiology of Cholecystitis and Gallstones and Their Production by the Intravenous Injection of Bacteria, J. Infect. Dis. **19**:527, 1916.

In the gallbladders showing slight changes 30 per cent. only yielded streptococci, in contrast to 75 per cent. in those showing marked changes. Moreover, the gallbladders in which marked changes existed showed the larger number of colonies. Some of these contained countless numbers of organisms, while those showing slight changes, with few exceptions, contained a small number. Of the latter, 58 per cent. gave no growth, while only 25 per cent. of those showing marked changes gave no growth. In the cases showing slight changes, colon bacilli were isolated in pure culture from 12 per cent. and in combination with streptococci from 6 per cent. The entire 15 per cent. of those with marked changes contained both colon bacilli and streptococci.

Some of the organisms, when first isolated, produced opaque, indifferent colonies on blood agar, and microscopically were grouped in diplococcus forms with little or no chain formation. Further study, however, proved them to be streptococci. In this connection an interesting observation was made. From one of these cases, showing a pure culture of opaque gray staphylococcus-like colonies, two strains derived from a single colony were studied. The one kept on blood agar alternately aerobically and anaerobically became a green-producing streptococcus. The other, planted alternately in dextrose brain broth and on aerobic and anaerobic blood agar slants, developed hemolytic powers.

TABLE 1.—RESULTS OF CULTURES

Material Cultured	Number	Per Cent. Showing		
		Streptococci	Colon Bacilli	No Growth
Gallbladders showing slight changes.....	50	30	18	58
Gallbladders showing marked changes...	20	75	15	25
Ulcers	4	100	0	0

The different strains varied somewhat in their fermentative powers. Of the eighteen studied, all fermented dextrose, lactose and maltose, three raffinose, four mannite, ten salicin and one inulin. One strain, after a single animal passage, had its fermentative powers changed, but it was still agglutinated, the same as the original strain.

Microscopic examination of the gallbladders failed to reveal bacteria when negative cultures were obtained, but bacteria were found consistently when the cultures were positive. Organisms were found in the lesions produced in rabbits, but were not found in normal tissue. At the suggestion of Dr. E. S. Judd, microscopic examination of liver sections which he removed were made in ten cases. Interlobular cirrhosis was found in six, no change in two, and a bile-duct involvement

in two. The livers which were normal and those showing fibrotic changes were found in cases in which the gallbladders showed marked and slight changes, while in those showing cholangitis there was little or no change.

ILLUSTRATIVE CASES AND ANIMAL EXPERIMENTS

Case 55.—A man aged 34 years, for the past two years had had gastric symptoms. Pains were aggravated by food, and soda gave no relief. He was operated on Aug. 20, 1918. The stomach was normal, the gallbladder showed slight changes and contained one large stone, the appendix showed slight changes; these two organs were removed. About 3 c.cm. of the fundus of the gallbladder was cultured.

August 21, all the cultures showed indifferent streptococci in pure form. Five c.c. of the dextrose brain broth culture was injected intravenously into Rabbit 1662.

August 22, the rabbit appeared to be well.

August 23, the rabbit appeared to be well; it was chloroformed, and the gallbladder was found greatly distended with watery bile; the walls were edematous. The stomach, spleen, kidneys, appendix, lungs and heart were normal. No other lesions could be found.

August 24, cultures made from the blood of the rabbit were negative, while those from the bile and gallbladder showed countless numbers of the injected streptococcus.

August 26, cultures were made from the pus expressed from the patient's tonsils.

August 27, the blood agar plate cultures showed indifferent and green-producing streptococci and colon bacilli. The dextrose brain broth cultures from the tonsil, containing both streptococci and colon bacilli, were injected intravenously into Rabbits 1682 and 1683.

August 28, both animals were found dead. Necropsy showed marked post-mortem changes but no evidence of specific localization. Five c.c. of the dextrose brain broth culture made from one of the indifferent colonies of streptococci was injected intravenously into Rabbit 1686.

August 29, the rabbit seemed well.

August 30, the rabbit seemed well; it was chloroformed, and the gallbladder was found edematous and distended. There were several small hemorrhages and white necrotic areas in the fundus. No other lesions were found.

August 31, cultures from the blood were negative. Cultures from the gallbladder showed streptococci in pure form. Microscopic examination of the gallbladder revealed streptococci in the tissues.

The primary cultures from three other cases of cholecystitis were injected intravenously in rabbits. The gallbladders in two of these showed marked changes and cholecystitis developed in each of the two rabbits injected. The streptococcus was recovered from the gallbladders in each, while the blood was sterile. The third strain isolated from a gallbladder having chronic changes showed no definite localization.

Case 58.—A man, 68 years of age, had had intermittent attacks of pain in the region of his stomach for the past thirty years. The pain was worse in the afternoon and was not affected by food. He was operated on Aug. 21, 1918. An ulcer, 1.5 cm. in diameter and 4 mm. deep, with markedly indurated walls, was found on the lesser curvature of the stomach about 5 inches from the pylorus. The ulcer was excised.

August 22, cultures of the emulsified ulcer showed streptococci in pure form. Five and 8 c.c. of the dextrose brain broth culture from the ulcer were injected intravenously into Rabbits 1675 and 1676, respectively.

August 23, Rabbit 1675 seemed well. Rabbit 1676 was found dead. The cardiac end of the stomach showed fourteen punctate hemorrhages with sur-

face erosion. There were no other lesions except coccidial abscesses in the liver and in the inguinal region.

August 24, cultures from the blood and bile were negative. Cultures from the lesions in the stomach gave streptococci in pure form. Rabbit 1675 seemed well. It was chloroformed, and the cardiac end of the stomach showed several punctate hemorrhages with beginning ulceration. Other lesions were absent.

August 25, cultures from the blood and bile gave no growth. Cultures from the affected areas of the stomach gave streptococci in pure form.

August 26, cultures were made from the pus expressed from the patient's tonsils.

August 27, the blood agar plate cultures contained colon bacilli and green-producing streptococci. Three c.c. of the dextrose brain broth culture containing colon bacilli and streptococci from the tonsils were injected intravenously into Rabbit 1681.

TABLE 2.—Results of Agglutination Experiment

Serums	Dilutions of Serums	Tonsil	Gallbladder	Ulcer	Ulcer	Ulcer
		Case 55	Case 55 After One Animal Passage	Case 58	Case 58 After One Animal Passage	Case 75
Case 55 (Cholecystitis)	1-2	0 cloudy	++	+	0	0
	1-4	++	++	+	0	0
	1-8	++	++	+	0	0
	1-20	0	++	0	0	0
	1-100	0	—	0	0	0
	1-500	0	0	0	0	0
Case 58 (Ulcer of Stomach)	1-2	0	++	+	++	0
	1-4	0	0	++	+++	++
	1-8	0	0	++	++	++
	1-20	0	0	++	+	0
	1-100	0	0	+	0	0
	1-500	0	0	+	0	0
Normal Control	1-2	0	0	+	++	0
	1-4	0	0	+	+	0
	1-8	0	0	+	0	0
	1-20	0	0	0	0	0
	1-100	0	0	0	0	0
	1-500	0	0	0	0	0
NaCl Control	1-1	0	0	0	0	0

August 28, the animal was found dead. There were marked postmortem changes, but no evidence of localization.

August 29, cultures from the bile were negative. Cultures from the blood showed countless numbers of green-producing streptococci.

One other rabbit was injected with streptococci from an ulcer. It showed definite lesions in the stomach as did the others. Cultures made from the lesions showed streptococci, while those from a normal portion of the stomach were negative.

To determine further the specificity of the organisms isolated, serums were obtained from the cases cited for agglutination purposes.

As shown in Table 2, the serum of the patient with cholecystitis agglutinated both the strains from the tonsil as isolated, and the strain from the gallbladder after one animal passage, but failed to agglutinate the ulcer strains. The serum of the patient with ulcer, on the other hand, agglutinated the homologous ulcer strain as isolated, and after one animal passage, and the strain from another case of ulcer, but not the cholecystitis strains. The normal human serum had little or no agglutinating power over any of the strains.

SUMMARY

By making cultures of the emulsified tissues of gallbladders or adjacent lymph glands, streptococci are found to be the chief microorganisms associated with cholecystitis. The direct etiologic relationship of the streptococcus is established by their presence, often in numbers proportionate to the degree of gross and microscopic changes, by their having elective affinity for the gallbladder of animals and by the specific agglutinating power of the serum of the patient from whom isolated. The elective affinity for the gallbladder of animals of the strains from the tonsils indicates strongly that cholecystitis is commonly a blood borne infection from a focal source.

THE PHYSIOLOGIC ACTION OF CANTHARIS*

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OMAHA

The article by S. T. Lipsitz[1] and collaborators on polycythemia induced by cantharis attracted our attention, inasmuch as it bore on a problem under consideration in this laboratory. Since the physiologic action of cantharides tincture has not been made clear by the work just referred to, and we could find nothing in the literature to elucidate this matter, we decided to perform experiments with this substance.

Lipsitz discovered in a case of acute cantharis poisoning which came under his observation a marked polycythemic condition. He and his collaborators have extended their investigation of this interesting phenomenon to animals. In this way they corroborated the observation that the administration of cantharis induces a lasting polycythemia. The question of the causation of this polycythemia, however, received no answer from their study. Several possibilities may be considered in this connection. The polycythemia may have resulted from vasomotor changes whereby an extra amount of erythrocytes from the deep organs is forced into the peripheral circulation. Thus, the appearance of polycythemia may be produced. That such an increase in the number of red cells through a redistribution of the formed elements of the blood sometimes occurs has actually been observed. Could the cantharis effect, which may last for days, also be due to a vasomotor reflex? The long persistence of the effect would probably be against this suggestion, but as we could find no information as to the influence of cantharis on blood pressure, we undertook several experiments with this in view.

A female dog weighing 9 kg. was used. The animal received 2 grains of morphin subcutaneously; then it was put under ether anesthesia, which was continued throughout the experiment. The blood pressure was recorded from the carotid artery. Five drops of cantharides tincture in Ringer's solution was injected into the external jugular vein. There was no effect on the blood pressure, but the respiration became more frequent. After five minutes another five drops of the tincture in Ringer's solution was injected, and five minutes

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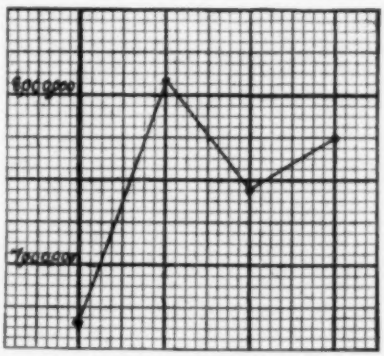
* From the physiological laboratory of the Creighton University College of Medicine.

1. Lipsitz, S. T., Fuerth, A. L., and Cross, A. J.: Polycythemia Induced by Tincture of Cantharides, Archives Int. Med. **20**:913, 1917.

later this was again repeated. No change in the blood pressure was obtained.

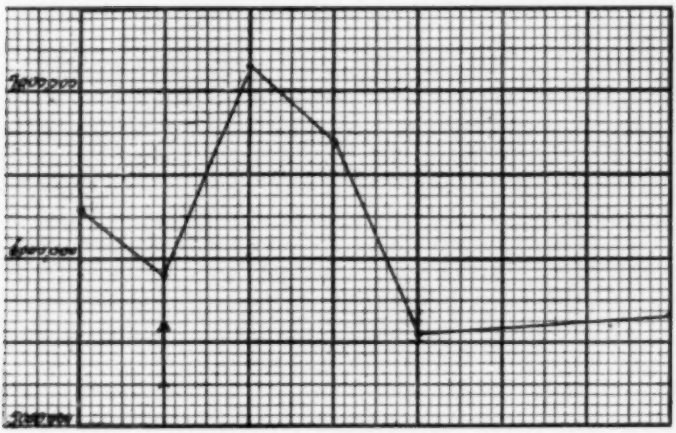
In another experiment with a male bulldog weighing 20 kg. we injected intravenously the equivalent of about 1 mg. of cantharidin per kilogram of body weight. (Computed from the fact that the tincture is 10 per cent. strong, and that the cantharis powder contains approximately 0.5 per cent. of the active principle.) The dog was etherized, and the carotid blood pressure as well as the respirations were graphically recorded. After a normal tracing had been obtained for several minutes, 40 c.c. of the tincture, made up to 100 c.c. with Ringer's solution, was injected into the femoral vein. The injection was made slowly, at the rate of 5 c.c. per minute. A very slight rise in pressure was observed at first. The respiration was very rapid and



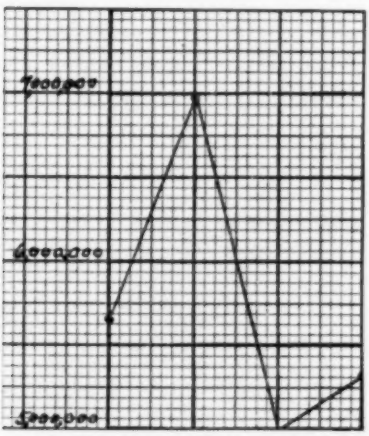
Experiment 1.—White rabbit; weight 7 pounds; normal; erythrocytes, 6,640,000; 25 drops of cantharides tincture were given by stomach tube: First day, erythrocytes, 8,072,000; second day, 7,440,000; third day, 7,766,000.

labored, and synchronous with the pulse. A distinct rattling sound in the chest could be heard. Twenty-five minutes after the injection the blood pressure remained practically normal.

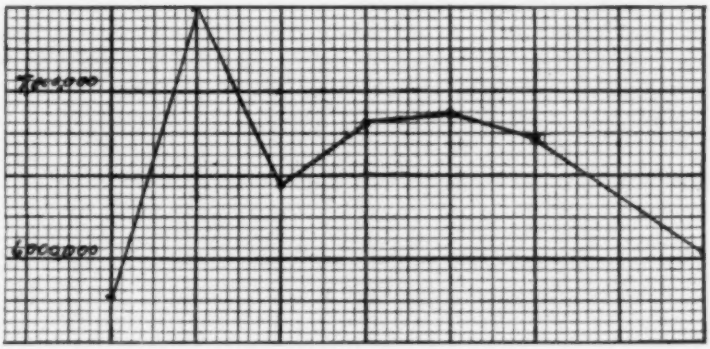
In a third experiment we used a male dog weighing 12.5 kg. The ether anesthesia was preceded by morphin. The blood pressure was recorded from the carotid. The cantharides tincture was injected into the external jugular. Twenty-five c.c. were injected at first, and fifteen minutes later this was followed by a similar dose. In other words, the equivalent of about 2 mg. of cantharidin per kilogram was injected into the blood. The carotid pressure remained normal for over an hour. Later it rose slightly above the orginal level. One hour and forty minutes after the injection the dog suddenly died.



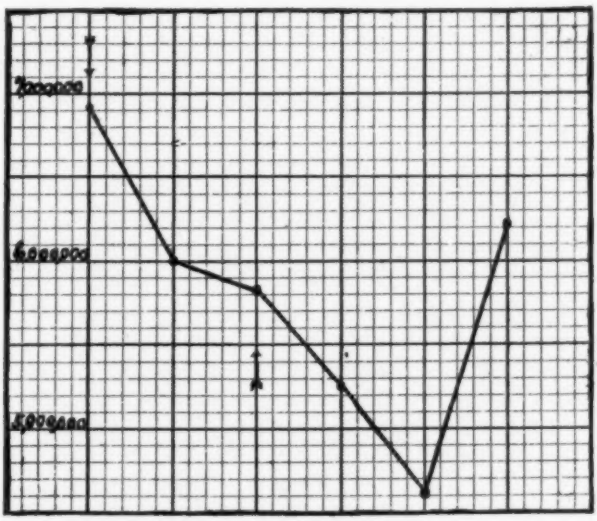
Experiment 2.—Black rabbit; weight, 5 pounds; normal; erythrocytes, 6,292,000; normal; erythrocytes, 5,906,000; 20 drops of cantharides tincture in 0.9 per cent. sodium chlorid solution injected into the vein of the ear: First day, erythrocytes, 7,140,000; second day, 6,692,000; third day, 5,542,000; sixth day, 5,650,000.



Experiment 3.—Black rabbit; normal; erythrocytes, 5,650,000; 1 c.c. of cantharides tincture in 0.9 per cent. sodium chlorid solution injected into vein of the ear: First day, erythrocytes, 6,975,000; second day, 4,963,000; third day, 5,313,000.



Experiment 4.—Black rabbit; weight, 4½ pounds; normal; erythrocytes, 5,763,000; 2 c.c. of cantharides tincture given by stomach tube: First day, erythrocytes, 7,500,000; second day, 6,450,000; third day, 6,812,000; fourth day, 6,875,000; fifth day, 6,713,000; seventh day, 6,038,000.



Experiment 5.—Same rabbit; normal; erythrocytes, 6,917,000; 1 c.c. of cantharides tincture injected into vein of the ear: First day, erythrocytes, 6,001,000; second day, 5,830,000; 2 c.c. of cantharides tincture given by stomach tube: third day, erythrocytes, 5,270,000; fourth day, 4,610,000; fifth day, 6,220,000.

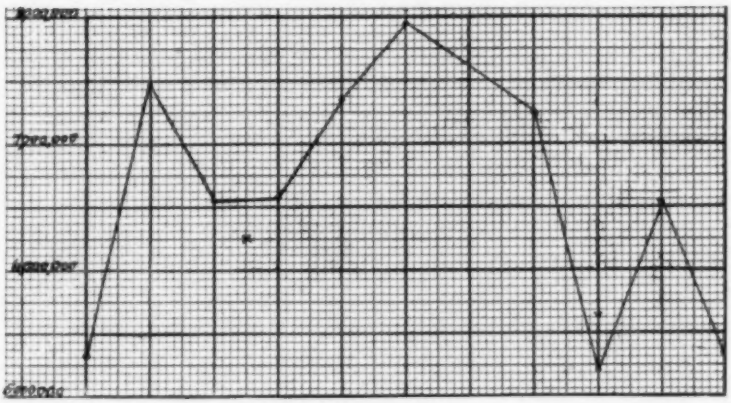
The necropsy findings were rather suggestive. The intestine, pancreas and spleen were grayish purple; the liver very dark and congested; the kidneys firm and strongly congested. The heart was in diastole; the veins were greatly distended with blood. The lungs were overfilled with blood, very hard and covered with dark purple patches.

Having thus failed to find any influence on the general blood pressure which might perhaps have explained the origin of the cantharis polycythemia, we turned our attention to another possibility, namely, the stimulation of the red cell-producing organs. We examined for this purpose specimens of blood from rabbits both before and after treating them with cantharides tincture. The blood was obtained by pricking the vessels of the ear. Smear preparations were made and the red and white count determined each time. To make the results strictly comparable, the same degree of dilution was always used.

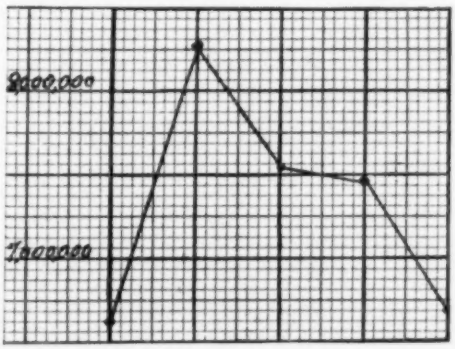
The stained preparations were carefully searched for evidence of new cell formation, and although dozens of slides were examined, we never found any normoblasts. In general, it may be said that the blood picture with the differential stain remained unchanged.

The leukocyte count proved of little or no consequence, but the enumeration of erythrocytes in our experiments with rabbits threw much light on the problem. In the following experiments the tincture of cantharides was administered to rabbits either by stomach tube or intravenously. The effect of these two modes of treatment was quite different, and this difference we think is significant in the interpretation of the cantharis "polycythemia." Before we record our experiments, however, we wish to point out that in our experience the cantharis is not nearly as toxic as it appears from Lipsitz' investigation. Our tincture was prepared from the best article on the market (Russian powdered cantharis) according to the United States pharmacopeia.

An examination of the data and curves just presented corroborate Lipsitz' observation of a lasting increase in the red cell count following the administration of cantharides tincture by stomach. It should be noted, however, that according to our experience the results following the administration of cantharis intravenously differ fundamentally from those occasioned by cantharis given by stomach. A glance at the curves shows this at once. Instead of a polycythemia lasting several days, the increase in the red cell count induced by an intravenous injection of cantharis is followed within forty-eight hours by a drop to the normal level or even below that. This striking difference in the durability of the effect on the blood is evidently associated with the rapidity of the elimination of the cantharis. Given by stomach it evidently acts as a gastro-intestinal irritant, being slowly absorbed and gradually eliminated through the kidneys. Introduced into the blood



Experiment 6.—Black rabbit; weight, 5¾ pounds; normal; erythrocytes, 5,315,000; 2 c.c. of cantharides tincture given by stomach tube: First day, erythrocytes, 7,470,000; second day, 6,550,000 (gave birth to two young); third day, 6,580,000; fourth day, 7,350,000; fifth day, 7,960,000; seventh day, 7,250,000; eighth day, 5,190,000 (1 c.c. injected into the vein of the ear); ninth day, erythrocytes, 6,540,000; tenth day, 5,350,000.



Experiment 7.—White rabbit; weight, 4½ pounds; normal; erythrocytes, 6,630,000; 2 c.c. of cantharides tincture given by stomach tube: First day, erythrocytes, 8,270,000; second day, 7,540,000; third day, 7,470,000; fourth day, 6,680,000.

the cantharis reaches the kidney by a more direct route, and is more quickly discarded. In both events, apparently, its elimination is associated with a loss of water, resulting in a concentration of the blood. The would-be polycythemia is therefore nothing but a state of blood concentration.

Experiment 5 suggests that rabbits may develop a certain immunity toward cantharis. This rabbit received cantharis four times, both intravenously and by stomach. When the tincture was given again intravenously a marked diminution in the erythrocyte number took place. The tincture was then given by stomach tube, but the red cell count continued to fall off. Thus from 6,917,000 per square millimeter the number diminished to 4,610,000 in five days. It then returned to the normal level again (6,220,000).

From our investigation we can conclude that in cantharis we do not possess a means of experimentally inducing polycythemia, inasmuch as it does not cause a production of new red cells, but merely occasions a condensation of the blood through the loss of water in the process of elimination of cantharis by the kidneys.

HYDROCEPHALUS AND CHOKED DISK IN DOGS*

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It can not be said that consideration of the subject of choked disk is a new thing, but, as long as the correct explanation of the origin of choked disk alone can give a satisfactory conception of its clinical significance, any additional data based on experimental observations may help to decide a question which already has called forth numerous discussions and an abundance of conflicting theories.

These discussions began with von Graefe, and since the appearance of his original article on this subject two principal theories have claimed most attention. According to one, choked disk finds its origin in local inflammatory reactions, while the other explains its occurrence on the basis of purely mechanical factors.

Historically, the mechanical theory is the oldest and was first advanced by von Graefe.[1] He believed that the increased intracranial pressure, acting by compression on the cavernous sinus, caused a stasis in the ophthalmic vein, which resulted in a choked disk. But, several years later, von Graefe saw the fallacy of this view when Seseman demonstrated the free anastomosis between the ophthalmic and facial veins. Therefore, the inability of the ophthalmic to empty into the cavernous sinus could no longer be considered as a cause.

The exceedingly rare occurrence of choked disk in cases of cavernous sinus thrombosis, as has been pointed out by Uhthoff,[2] and the experimental demonstration that even extreme direct compression of the sinus is insufficient to induce the appearance of a swollen disk, is additional proof that its pathogenesis does not lie in a failure of the retinal and optic veins to empty into the cavernous sinus.

Such factors as these led Schmidt-Rimpler,[3] after Schwalbe[4] had shown that the intravaginal lymph spaces of the optic nerve communicate with the subarachnoid space of the brain, to suggest a direct driving of cerebrospinal fluid through the lamina cribrosa with an edematous infiltration of the nerve head as a consequence.

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* Contribution 65 from the Departments of Anatomy and Pharmacology, Northwestern University Medical School, Chicago.

1. Von Graefe: Arch. f. Ophthal. **7**:56, 1860.
2. Uhthoff: Neurol. Centralbl. **23**:903, 1904.
3. Schmidt-Rimpler: Arch. f. Ophthal. **15**:193, 1869.
4. Schwalbe: Arch. mikr. Anat. **6**:1, 1869.

This meant that a hydrops of the nerve sheath existed, and Manz[5] argued that since this sheath was under tension, it caused compression of the optic nerve vessels as they pass through the lamina cribrosa and that this would result in an edema of the disk.

This view, which regards the rise in intracranial pressure as the primary factor, and possibly acting through compression on the vessels as they pass through the lamina cribrosa or nerve, has found adherents in Cushing and Bordley,[6] and is by many considered as the most satisfactory explanation offered so far.

The number of those who regard choked disk as a manifestation of an inflammatory process has steadily decreased. Toxic substances, the product of tumor cells or of inflammatory processes in the meninges, were thought to be carried by the blood or lymph stream to the optic disk and there caused a local reaction which made itself apparent in a swollen disk. With slight variations these views found adherents in Gowers,[7] Leber,[8] Deutschman,[9] Elschning,[10] Lawford and Edmunds[11] and others.

But, within the last few years the inflammatory theory has been accepted by few as a satisfactory explanation. This is especially true since Paton and Holmes[12] have published their histologic studies of postmortem findings in several hundred cases of choked disk occurring in association with cerebral tumors. They report that in the early stages a simple edema without round cells is the prominent feature. Even in cases of long standing the characteristics present are not those of an inflammatory process. If any inflammation exists the picture is such that it must be considered as merely secondary to the disturbance produced by the edema.

These findings of Paton and Holmes on such abundant clinical material have been corroborated by similar pictures in experimental animals. They may, therefore, be considered as quite conclusive evidence against the theory that choked disk, in tumor cases at least, is due to an inflammatory reaction; whereas the importance of increased intracranial pressure stands out prominently. The full meaning of this was recognized by Parsons,[13] who says: "All those who have had opportunity to watch the extraordinary effect of the relief of intra-

5. Manz: Deutsch. Arch. f. klin. Med. **9**:339, 1872.
6. Cushing and Bordley: J. A. M. A. **52**:353, 1909.
7. Gowers: Medical Ophthalmoscopy. London, 1879.
8. Leber: Tr. First Internat. Cong., London **3**:138, 1881.
9. Deutschman: Neurol. Centralbl. **23**:673, 1904.
10. Elschning: Arch. f. Ophthal. **41**:179, 1895.
11. Lawford and Edmunds: Tr. Ophthal. Soc. **3**:138, 1883.
12. Paton and Holmes: Brain **33**:389.
13. Parsons, J. H.: The Pathology of the Eye, **2**:1350, 1908.

cranial pressure on a choked disk must agree that no theory which leaves this element out of account requires further consideration."

Numerous attempts have been made to sift the conflicting facts and theories of the origin of choked disk by experimental studies. A sudden increase of intracranial tension was always an essential feature in the methods employed.

Manz,[14] one of the first of the experimental workers on this subject, noted that fluids injected into the subdural space passed into the nerve sheath. An engorgement of the retinal veins and a transient swelling of the optic disk resulted from this.

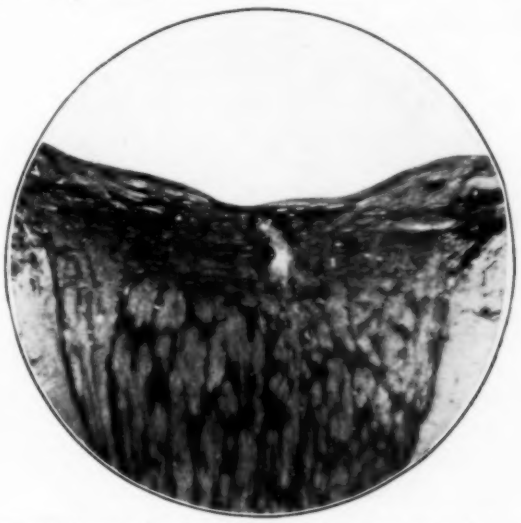

Fig. 1.—Photomicrograph of disk and optic nerve of a dog. Normal.

While Manz was unable to demonstrate a permanent choked disk by this same method, Schieck[15] succeeded and produced a definite optic disk swelling. However, when he placed sponge tents under the skull or created artificial tumors through the subdural injection of paraffin, he failed. From this he concluded that a mere rise in intracranial pressure is insufficient and that an increased amount of cerebrospinal fluid is an essential.

Levinsohn[16] observed a venous hyperemia and some retinal hemorrhages after injecting fluid, but did not find any evidence that the high tensioned cerebrospinal fluid permeated through to the vaginal sheath spaces.

14. Manz: München. med. Wchnschr. **35**:531, 1888.
15. Schieck: Die Genese der Stauungspapille, Weisbaden, 1910.
16. Levinsohn: Ber. d. Ophth. Gesellsch., Heidelberg, 1911.

With fluid injected under considerable pressure into the subdural space, Cushing and Bordley[17] produced an acute edematous swelling of the nerve head and retina. Digital pressure when exerted for a period of several minutes against the dura and also elastic foreign bodies when placed between the skull and dura caused a like optic picture.

Parker,[18] within the last years, published findings based on a difference in tension in the two eyes and a subsequent creation of intracranial pressure produced by the usual methods; but the most satisfactory results were obtained by the use of sponge tents. His experiments were carried out on dogs and monkeys with identical results. He found that the eye that possessed the least tension is the first to show choked disk.

Fig. 2.—Section of choked disk, right eye, Dog 2.

It is evident from the foregoing summary of these experimental methods that they have in the main caused a sudden increase in cerebral pressure. This created conditions which resembled such as are present in sudden hemorrhages and edema of the brain and in no ways are comparable to the gradual process which accompanies the growth of cerebral tumors or development of hydrocephalus. Any new experimental work, therefore, which attempts to explain the cause of choked disk should create conditions that will cause the same gradual changes

17. Cushing and Bordley: Bull. Johns Hopkins Hosp. **20**:95, 1909.
18. Parker: J. A. M. A. **17**:1052, 1916.

that occur and are observed clinically. In the hope that this could be attained with the following method and that new information might result the present work was undertaken.

EXPERIMENTAL METHODS AND OBSERVATIONS

Because of the difference in the optic blood supply between canines and primates, the results obtained through experimentation on dogs by other workers on this subject have frequently been held as being nonapplicable to man. On account of this criticism it was decided to create a condition the existence of which in man, namely, hydrocephalus, is known to be accompanied almost always by choked disk.

Fig. 3.—Section of choked disk, left eye, Dog 2.

To induce the formation of hydrocephalus the aqueduct of Sylvius was blocked with soft paraffin. The paraffin had a melting point ranging around 42 C., and was only used at a temperature just sufficient to prevent hardening while in the equally warmed syringe.

After several experimental attempts it was found that a small trephine opening, when placed 6 mm. above the highest nuchal line and 5 mm. to the right or left of a line carried forward from the center of the external occipital protuberance, would permit a needle to pass just in front of the tentorium cerebelli. By this route it is possible to be guided by the bony tentorium and damage to the longitudinal sinus is easily avoided. Since the lower edge of the tentorium practically touches the upper surface of the pons, it is necessary to

depress the needle only 1.5 or 2 mm. more before the point enters the aqueduct. It should be remembered that the needle does not enter in an exact vertical direction, but must descend somewhat obliquely toward the midline. This work was carried out under strict aseptic precautions, and about 1 c.c. of sterilized paraffin injected.

It is necessary to mention here that some authors speak of trephining as synonymous with decompression and therefore may attempt to explain the regularly occurring delay in the development of hydrocephalus in these animals as due to this opening in the skull. The trephine opening, however, was never more than 2 mm. in diameter, and the dura was never perforated except with the fairly thin needle of the paraffin injection syringe. It is a matter of surgical knowledge

Fig. 4.—Section of choked disk, left eye, Dog 6.

that the opening in the skull must be larger than that made by a common trephine when relief of pressure is sought by decompression, and in addition the dura must be incised.

To indicate fully the manner in which the experiments were carried on the following two are given in detail. These are typical, and an account of any of the others would be mere repetition.

Observation 2.—Oct. 10, 1916. Medium sized, black fox terrier. Ether anesthesia.

Ophthalmoscopic examination of fundus shows nothing abnormal. Vessels appear normal and optic disk is on same plane as the retina.

1:30 p. m. Small trephine opening made and 1 c.c. of paraffin injected into the aqueduct. Retinal vessels and disk show no change.

2 p. m. and 3 p. m. No changes noted.

October 11. Dog is lively and normal to all appearances. Ophthalmoscopic examination reveals no change from picture presented yesterday.

October 12. Dog is normal and active. The slight skin incision over the occipital protuberance has united. Suture clip is removed. The ophthalmoscope shows very prominent and tortuous retinal veins but no swelling of the optic disk.

October 13. Dog appears apathetic and refuses food. Fundus oculi show prominent and tortuous veins with disk swollen 2 D in left eye and a little more than 1 D in right eye.

October 14. No ocular examination made. Animal keeper reported that dog was apathetic and refused food.

October 15. Dog is extremely stuporous. Moves only when urged. Movements are spastic. Eyes show a distinct exophthalmos. Ophthalmoscope shows presence of retinal hemorrhages and a high choked disk in both eyes. Right eye 5 D; left eye 4 D. Animal is chloroformed.

Fig. 5.—Section of choked disk, right eye, Dog 6.

Necropsy.—Removal of the occipital skull reveals a tense dura. On incision a clear fluid which is under considerable pressure escapes. The amount is slight. The brain sulci are indistinct and appear flattened from pressure against the dura.

The optic nerve is firmly lodged in the bony foramen. The sheath appears to have a larger than normal diameter, is sacculated and on incision fluid escapes from it. The eyes and optic nerves were fixed in Perenyi's fluid.

The brain was hardened in 10 per cent. formaldehyd solution and after several days division of it along the longitudinal fissure showed a small plug of paraffin lodged securely in the aqueduct and extending anteriorly into the third ventricle and posteriorly against the cerebellum as it arches over the fourth ventricle. No evidences of local inflammation are present.

The third and lateral ventricles are slightly distended but no prominent hydrocephalus exists. This is to some extent due to the shrinkage resulting from the fixation, as the brain now no longer bulges along the lateral ventricles as it did in the fresh state when an acute ventricular distention was present.

Histologic Observations.—Histologic examination of the eyes shows a distinct swelling of the disk, illustrated in Figures 2 and 3. Definite separation by edema of the nerve fibers as they pass through the disk and over the retina is apparent. No other inflammatory signs such as round cells are observed. Numerous extravasations of blood occur over the entire retina.

Observation 6.—Jan. 3, 1917. Small mongrel dog. Ether anesthesia.

Retinal vessels and disk are normal. The skull is trephined as usual and the aqueduct occluded. About 1 c.c. of paraffin is injected. This operation has no effect on the retina or disk.

January 4 to 7. Dog is lively, feeds well and appears normal in all respects.

January 8. Dog is listless, feeds poorly. Ophthalmoscopic examination shows tortuous and prominent retinal vessels. Disks are slightly swollen.

Fig. 6.—Section through the center of optic nerve and papilla from a dog killed three days after the aqueduct had been plugged. Vascular congestion of retina and dilatation of the dural sheath are present. The subarachnoid spaces are not distended. The venous engorgement, due to shrinkage in fixation, is not as prominent as it presented itself in the live animal.

January 9. No observations made.

January 10. Dog has a definite exophthalmos. Retinal vessels do not stand above retina with the prominence observed two days previously. Small hemorrhagic areas are scattered over entire retina. Left eye has a choked disk that measures 7 D.; right eye between 5 and 6 D. On this day the animal was extremely stuporous, refused to walk and when forced showed a marked spasticity. Later in the day he became comatose and was chloroformed.

Necropsy.—Both eyes and optic nerves were fixed immediately.

Opening the skull showed conditions identical with those observed in Dog 2. The brain was fixed in 10 per cent. formaldehyd solution and subsequent examination of it showed no variation in any important detail from the findings described in Observation 2.

Histologic Observations.—No marked variations in detail exist from those mentioned under Dog 2. The vascular congestion, with the wide spread edema and absence of round cells are the prominent features. Photomicrographs of both eyes and optic nerves are given in Figures 4 and 5. These photomicrographs are of the same magnification as that of the normal eye, Figure 1.

The foregoing two detailed accounts, for the sake of space, must suffice to illustrate the type of experiments carried on. However, out of a series of ten dogs, six developed the conditions mentioned within a period never shorter than three days nor longer than ten days after

Fig. 7.—Moderate power photomicrograph of a choked disk retina to illustrate the extent of the edema among the nerve fibers. This is taken from the same respective location in the retina as Figure 8. Notice the difference in the thickness of the optic nerve fiber layer and the extent to which it is filled with edema. The internal limiting membrane is raised and in folds. Taken from right eye of Dog 4.

the aqueduct had been blocked. Necropsies on the four animals that did not develop choked disk showed that the aqueduct had not been completely occluded. This was due in two instances to an insufficient amount of paraffin, in another it had entered the third ventricle, and in the fourth it had lodged above the aqueduct.

It was noticed in every animal that developed an acute hydrocephalus that the first sign of an increased cerebral pressure was always a greater prominence of the retinal vessels, and especially the veins. This was observed as early as the second day, more frequently, however, on the third day, and always from twelve to twenty-four

hours before any swelling of the optic disk appeared. As these vessels became increasingly prominent their tortuosity increased and stood out boldly above the surface of the retina.

On account of these observations several animals were killed within three or four days after the aqueduct had been plugged at a time when the retinal venous engorgement was prominent, but signs of actual swelling of the disk did not as yet exist. This was done to determine whether such venous engorgement could possibly be associated with some changes within the sheath of the optic nerve itself.

The necropsies on these animals gave uniform results. In all a mild degree of hydrocephalus existed. The brain was fairly tense, as though slight pressure was exerted from within. The sulci, however, were still plainly marked and the brain was not pressed so firmly against the dura as in the case of those animals that were allowed to develop a severe hydrocephalus.

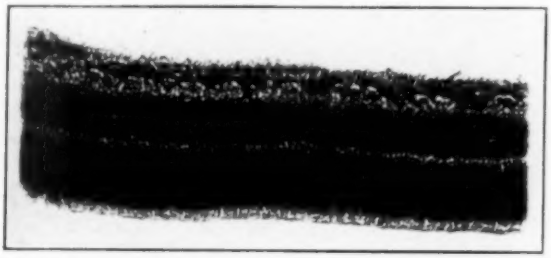

Fig. 8.—Photomicrograph of a normal retina to contrast with Figure 7. The magnification is the same. Note the difference in the thickness of the nerve fiber layer.

Histologic examination of the eyes shows prominent retinal vessels; evidence of retinal hemorrhage is absent. Swelling of the disk is not apparent, nor can traces of edema be found among the optic fibers of the retina. The dural portion of the optic sheath is moderately distended, but sacculations, such as occurred in the severe grades of hydrocephalus, are absent, nor are there indications of distended subarachnoid spaces, Figure 6.

It is apparent from these experiments that a condition of hydrocephalus can be induced in an adult dog by plugging the aqueduct of Sylvius with paraffin, and that where such condition becomes established, choked disk will result. But it will also be noticed that the development of such an hydrocephalic condition is a gradual process which requires at least seventy-two hours before it will manifest itself through a retinal venous engorgement. In none of these animals did this process develop into a severe hydrocephalus, associated with

choked disk and a subsequent comatose condition, in less than ninety-six hours. These results are at variance with the observations of Frazier and Peet[19] who induced the same condition within a period of twenty-four to thirty-six hours by injecting aleuronat suspension into the third ventricle and placing a gauze plug against the lower end of the aqueduct.

The histologic preparations of these eyes show that the disk undergoes severe changes after the animal has suffered from hydrocephalus for a period of three to five days. An edema is present not only in the disk, but throughout the entire nerve fiber layer as it speads over the retina. The fibers are separated and the spaces that formed are filled with fluid. In the advanced stages a stretching and separation of the fibers extends even into the anterior layers of the lamina cribrosa. As a result of this infiltration of fluid the disk is uniformly increased in size and spreads out over the retina.

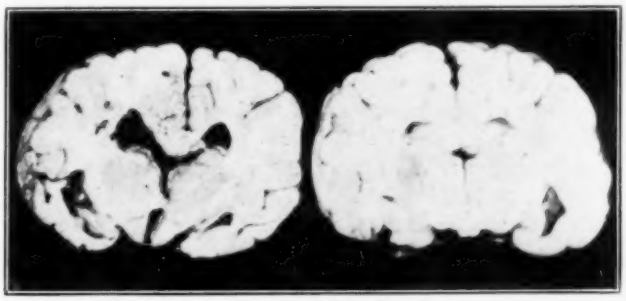

Fig. 9.—Coronal section of two forebrains. A, hydrocephalic brain to contrast with B, which is a normal dog's brain; seven-eighths natural size.

This widespread edema through the nerve fibers of the disk and retina causes considerable disturbance. Frequently the course of these fibers becomes S-shaped in outline. This is a feature to which Paton and Holmes have already called attention in their observations on clinical material.

The histologic findings in these experiments agree with those obtained by other workers. Round cells, such as would be expected to be present were this condition a true inflammation, are absent. Instead, a clear noncellular fluid is easily demonstrated between the fibers (Fig. 7), giving in all respects the picture of a simple edema. In these hydrocephalic dogs a choked disk with the histologic picture of a true inflammation did not exist, and it may therefore be well assumed that it was noninflammatory in origin.

19. Frazier and Peet: Am. J. Physiol. **35**:268, 1914.

It was noted in every instance where a pronounced choked disk occurred that at least a moderate ventricular distention (Fig. 9) was present. This distention was never extreme, which may readily be explained on the basis of the rigidity of the skull.

Recently Tilney[20] called attention to the presence of a small canal extending out from the third ventricle above the optic nerve and chisma. This tubular extension from the third ventricle persists into adult animals only in its proximal portion, and according to Tilney extends along the nerve for a distance of 0.75 mm.

It is Tilney's opinion that this supra-optic canal is of clinical significance[21] in connection with choked disk where there is an accumulaton and retention of fluid in the forebrain ventricles. With this idea in mind several of these moderately distended brains were sectioned serially through the region of the optic chiasma in the hope that the ventricular dilatation had caused a distention of the supraoptic canal. Nothing definite could be established, on the basis of this material, at least. In consequence the hypothesis that choked disk is possibly the result of distention and transmission of fluid through a possible supra-optic duct finds no corroboration here.

Attention has already been called to the uniform occurrence of a moderately distended dural sheath space (Fig. 6) at a time when nothing more than a retinal venous engorgement marked the development of an internal hydrocephalus. As this last condition progressed the sheath distention became more prominent until it showed frequent sacculations (Fig. 12) and involved the subarachnoid spaces. This same condition has been observed on clinical material by Paton and Holmes in England, and by Cushing and Bordley in this country. It is evident, therefore, that hydrocephalus in a dog will lead to the same conditions that present themselves in man suffering from ventricular distention as a result of tumor growth or of some other cause.

With this established, no great recognition must be given to the anatomic difference present in the circulation of the eye of man and that of the dog. By many this has been held as sufficiently important to vitiate the application to man of the experimental results obtained on dogs. Particularly so, since in the dog only a very small amount of blood returns from the eye into the cavernous sinus, leaving the greatest portion to flow through the vorticose and anterior ciliary veins into the external jugular. In consequence, the venous circulation of the dog's eye is essentially extracranial, while in man it is primarily an intracranial return system through the central vein into the cavernous sinus. True, an anastomosis occurs between the central and

20. Tilney: J. Comp. Neurol. **25**:213, 1915.
21. Tilney: Anat. Record **10**:250, 1916.

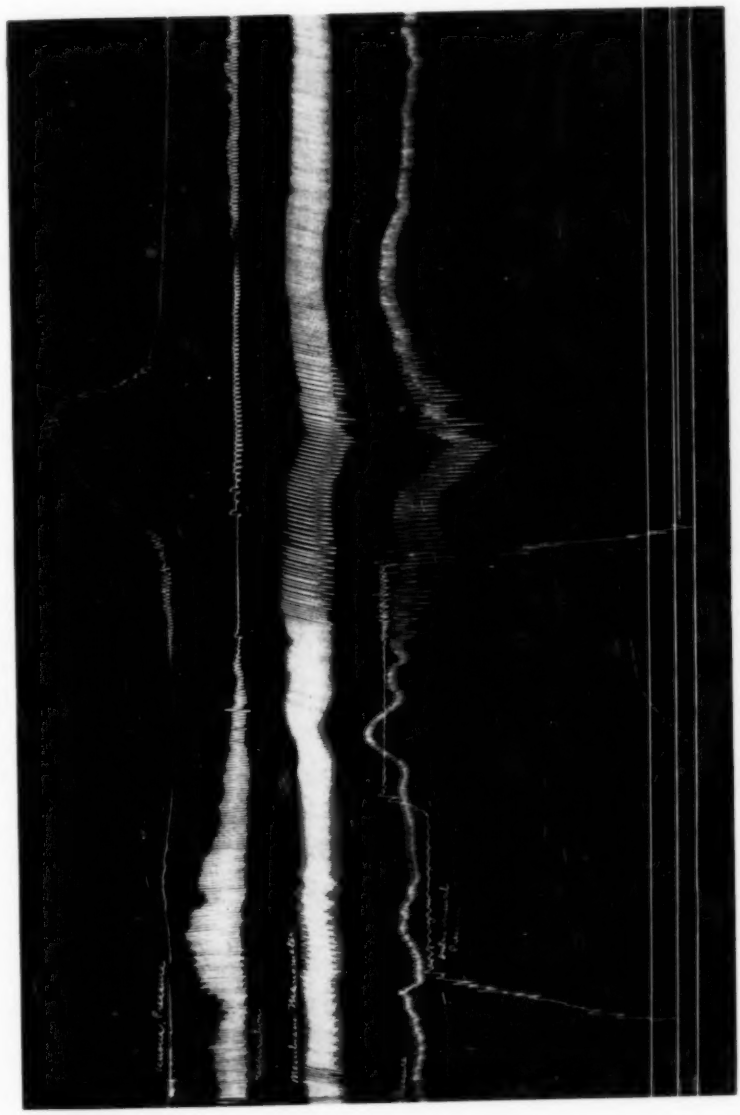

Fig. 10.—A graphic representation of the effect of increased intracranial pressure on the venous and arterial pressure, on respiration and the heart rate. The animal was under ether anesthesia only. Observation made on Dog 6, Series II.

ophthalmic veins, which provides, however, only for an emergency flow through the facial into the external jugular. Due consideration of these enumerated differences, combined with a recognition of the similarity of optic changes produced in man and in dogs by an hydrocephalus, will lead to the conclusion that no great importance can be ascribed to the local anatomy of the circulation of the eyes as a causative factor in the origin of choked disk.

That the physiology of that circulation is, however, a very important factor in the production of optic disk edema is constantly indicated by several conditions: These are, first, the early and uniform appearance of a retinal venous engorgement, which occurs equally in animals with an intracranial or extracranial return circulation; second, the extension of this venous engorgement to the peripheral system so that it becomes especially marked in the conjunctival and anterior ciliary veins; third, the development of a distinct and frequently an extreme exophthalmos, accompanied by a marked edema of the tissues within the optic orbit; lastly, by the results of physiologic and pharmocologic experiments undertaken in order to control these observations.

These experiments were carried out on eleven dogs under the direction and kind assistance of Professor Becht of the pharmacology department. I take great pleasure in acknowledging my thanks to Professor Becht for his interest in this phase of the work and his personal assistance in performing many of the following experiments.

An acute hydrocephalus was produced in these animals by allowing fluid under pressure to enter the subdural space. This method needs no further description, since it has been used by many experimenters. The typical notes of such an experiment are as follow:

Observation 4.—Ether anesthesia. Brown fox terrier.

1 p. m. The skull was trephined and a tightly fitting cannula placed over right hemisphere and connected with pressure bottle filled with warm normal saline solution. Tubing from the pressure bottle was also connected by means of a "T" to a mercury manometer to record the pressure. The right carotid artery was exposed, a cannula inserted and connected with another mercury manometer to obtain records of the blood pressure and heart rate. The axillary vein was next exposed and the venous pressure taken from here by means of a manometer containing sodium carbonate solution. From this the venous pressure could be read off directly, but to obtain a permanent and graphic record the manometer was connected to a tambour and tracings were made of this simultaneously with the blood and intracranial pressure.

1:40 p. m. Examination of the eyes shows nothing abnormal. The retinal vessels are normal in size and color. The disk is on the same plane with the retina.

1:50 p. m. Fluid is allowed to enter the subdural space. The pressure was gradually raised to equal that of the arterial pressure. Within a minute the effect of this became visible in a marked slowing of the heart, a transitory rise and then a fall in blood pressure. This was accompanied by a marked rise in venous pressure. The intracranial pressure was now maintained at this level. Observations on the fundus oculi showed veins which were quite

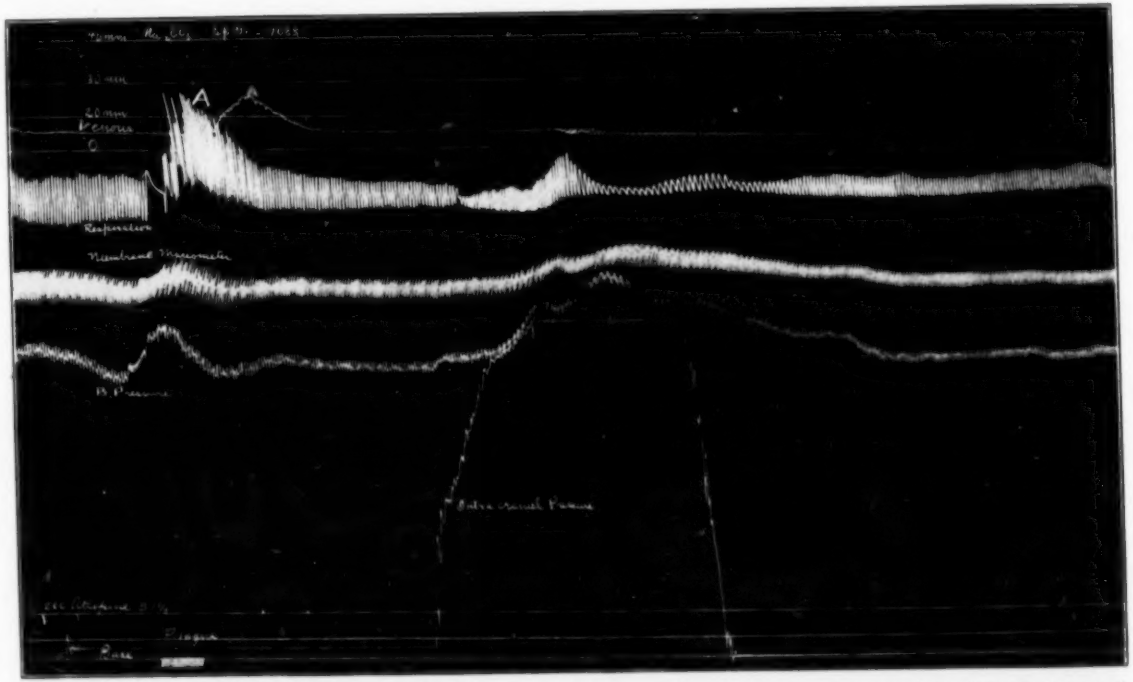

Fig. 11.—A graphic illustration of the failure to obtain any rise in venous pressure following an increased intracranial pressure, after the animal had received 2 c.c. of a 0.1 per cent. solution atropin sulphate. The rise in venous pressure at A is due to electrical stimulation of the vagus within a minute after the atropin had been injected.

tortuous and very prominent, but no signs of a swollen disk presented themselves.

Respiration stopped for a period of a minute and then broke through again. The venous pressure dropped slightly but was still high. Blood pressure remained decreased.

2: 10 p. m. Venous pressure is still high; 45 mm. of water. Retinal veins are tortuous and disks are swollen in both eyes.

2: 30 p. m. Intracranial pressure is still maintained at the former level. Respiration is slow. Blood pressure is decreased but venous pressure rises higher. Now up to 60 mm. of water. Both eyes show a distinct swollen disk, about 3 D. in left and 2 D. in right. A definite exophthalmos is present. Conjunctival veins are swollen. Also the anterior ciliary. The conjunctivae are edematous. The pupils are widely dilated. Pressure on the eyeball reduces the tortuosity and size of the veins, but no decided influence on the disk is observed.

3 p. m. Venous pressure is still high. No further eye changes but the exophthalmos has become most pronounced. Both eyes bulge far beyond their orbits. A marked palpable increase in intra-ocular tension exists.

3: 30 p. m. Intracranial pressure is maintained at the original level of 140 mm. Hg. Venous pressure rises rapidly, while there is an equally rapid drop in arterial pressure. Respiration is very slow and failing.

3: 45 p. m. Respiration has failed and heart stops beating within a minute. Both eyes and optic nerves are fixed immediately for histologic study.

As the intracranial pressure approached or went above the height of the blood pressure in these animals a drop in the heart rate and of the blood pressure and a rise in venous pressure occurred. This rise in venous pressure was not a local one, but was general, as is indicated by the identical results, whether the pressure was taken from the axillary or from the femoral vein.

A study of the tracings of this series of experiments showed that the increased intracranial pressure always resulted in a marked slowing of the heart rate. This slowing of the heart rate was uniformly accompanied by a progressive rise in venous pressure and subsequent to this definite signs of choked disk would develop.

On the basis of these observations a second type of experiments was decided on. In these the inhibitory action of the vagus on the heart was removed by atropin in order to determine whether the prevention of a high venous pressure in the presence of an increased intracranial pressure might possibly also prevent the formation of a choked disk.

A series of three such experiments was conducted. These in the details of procedure were duplicates of the foregoing except that the animals were thoroughly atropinized by the hypodermic injection of 5 c.c. of a 0.1 per cent. solution of atropin sulphate.

In these animals the intracranial pressure could easily be raised to an equal level with that of the arterial pressure and even above without a resultant rise in venous pressure. Instead of a rise in venous pressure there followed, however, an increase in heart rate and always a rise in arterial pressure.

Observations made on the eyegrounds of these atropinized animals during the course of the experiments showed that the continued high intracranial pressure had no effect on changing the appearance of the retinal veins or in producing a choked disk. No sign of exophthalmos ever developed, nor were there any visibly congested anterior ciliary or conjunctival veins in any of these atropinized animals.

The high intracranial pressure was maintained for as long a period as three and one-half hours in two of these dogs, and no variation from the normal occurred in their retinal veins or in the disk itself until just before their death, when the heart action became feeble. During the last half hour a slight retinal venous engorgement became apparent, but at no time did an actual choked disk develop which was visible ophthalmoscopically or afterwards in the histologic preparations of the eyes from these dogs.

TABLE 1.—To Illustrate the Difference in the Effect of Increased Intracranial Pressure on the Venous and Arterial Pressure of Three Atropin-Free Against Three Heavily Atropinized Dogs *

Ether Anesthesia

Dogs: Atropin-free				Dogs: Atropinized with 5 C.c. of A 0.1% Solution			
Dog No.	C. S. F. P., Mm. Hg	Venous P., Mm. H_2O	Arterial P., Mm. Hg	Dog No.	C. S. F. P., Mm. Hg	Venous P., Mm. H_2O	Arterial P., Mm. Hg
2	Normal	2.10	114.0	7	Normal	3.4	130.0
	+156	70.0	80.0		+128	3.2	148.0
3	Normal	4.0	112.0	8	Normal	4.1	132.0
	+160	52.0	88.0		+148	3.8	140.0
4	Normal	4.5	116.0	9	Normal	1.4	120.0
	+126	65.0	120.0		+210	1.8	206.0

* Dogs 1 and 5 are not included in this series because of mechanical errors which occurred during the experiment. Dog 6 is listed separately in Table 2 and Dogs 10 and 11 in Table 3.

Table 1 will illustrate the marked difference in effect of increased intracranial pressure on the arterial and venous pressures when the accentuated inhibitory action of the vagus on the heart is allowed to act and when the same is removed by the use of atropin. The difference in the venous pressures is extremely marked and to bring this particularly to the attention of the reader a summary of the various tracings from six experiments is given in the table.

It must suffice at this time to draw the attention of the reader to a close comparison of the figures given in Table 1. Their full significance will be discussed later along with the results of experiments in which the effect of increased intracranial pressure was studied in animals which were for the first part of the experiments atropin-free

and for the remaining part heavily atropinized. The conduct of these experiments was the same as that of the foregoing, except that after a complete record of the effect of increased intracranial pressure exerted for only a brief period had been obtained this same animal was given atropin and the intracranial pressure was again raised to the former level. A graphic record of this is given in Figures 10 and 11. This experiment represents a combination of the former two types the results of which are given in Table 1, and therefore Figures 10 and 11 also serve to illustrate these graphically.

For comparing the effect of such increased intracranial pressure on the arterial and venous pressure of the same dog when atropin free and when atropinized, Table 2 is given. It is based on the results from Dog 2, which are graphically given in Figures 10 and 11.

TABLE 2.—Based on Experimental Results from Dog 6, Illustrating the Influence of Atropin on the Effect of Increased Intracranial Pressure

Ether Anesthesia

Before Atropin					After 2 C.c. of 0.1 per Cent. Solution of Atropin				
Intracranial Pressure	Venous Pressure, Mm. of H_2O	Blood Pressure, Mm. of Hg	Heart Rate, per Min.	Respiratory Rate, per Min.	Intracranial Pressure	Venous Pressure, Mm. of H_2O	Blood Pressure, Mm. of Hg	Heart Rate, per Min.	Respiratory Rate, per Min.
Normal	4.1	132	104	60	Normal	4.05	142	90	45
+142 mm.	51.56*	108	30	10	+154 mm.	4.0*	178	110	24
Normal	4.3	134	85	54	Normal	3.5	138	92	45

* Contrast venous pressure before atropin is given with the same after atropin.

A full discussion of all of the figures in this table is not necessary. It is important to notice, however, that in this animal, as also in the three listed in Table 1 which were atropin free, the arterial and venous pressures undergo a marked change. Reference to Figure 10 shows that there is first a slowing of the heart rate, and this is soon accompanied by a fall in arterial pressure which is prelimniary to a rise in venous pressure. Release of the intracranial pressure results soon in the former vascular equilibrium.

Contrast these findings now with those given in the last half of Table 2 and illustrated graphically in Figure 11 as representing the effect of even a higher increase in intracranial pressure on the heart rate and on the arterial and venous pressures. Here the heart rate steadily increased, the arterial pressure rose and the venous pressure remained practically the same. If any favoritism was shown in the way of a less high intracranial pressure it was in this as in the other dogs listed in Table 1 for those without atropin.

Now, inasmuch as none of these atropinized dogs showed any signs of a choked disk, whereas it was uniformly obtained in the atropin-free dogs suffering from similar or less pressure, it does not seem too much to say that the existence of a high venous pressure is an important factor in the formation of optic disk edema. This statement is based on the fact that in these experimental animals which were subjected to an increased intracranial pressure and in which by the use of atropin the inhibitory action of the vagus was removed, a failure in the production of a high venous pressure and of any visible evidence either ophthalmoscopically or histologically of a choked disk resulted; while, on the other hand, in those animals in which the vagus action was not removed by atropin such, or even less, increased intracranial pressure uniformly resulted in a high venous pressure and pronounced optic disk edema.

TABLE 3.—Ophthalmoscopic Observations on Dogs 10 and 11 *

Dog 10 Without Atropin	Dog 11 Atropinized
1:30 p. m. Retinal vessels and optic disk normal; pulse 102	R. V. and optic disk are negative; pulse 120
1:40 p. m. Pressure applied to both dogs	
1:50 p. m. R. V.† quite tortuous and raised; no optic disk edema; pulse +90	R. V. are negative; pulse 130
2:10 p. m. R. V. very tortuous; disk appears slightly changed but no definite swelling; pulse 80	No changes; pulse 150
2:30 p. m. Choked disk in both eyes; right eye 2 D.; left eye 1 D.; pulse 72	No changes; pulse 150
3:00 p. m. Choked disk has increased on both sides to 3 D.; beginning exophthalmos present; pulse 70	No changes; pulse 140
3:30 p. m. Disk now raised 4 D. both eyes; marked exophthalmos	No changes; pulse 126; no exophthalmos
4:00 p. m. No changes from foregoing; pulse 68	No changes; pulse 120

* The intracranial pressure was raised within ten minutes to 140 mm. of Hg and maintained there for four hours.
† R. V. = retinal vessels.

It is apparent, then, that two factors are necessary in the production of choked disk. These are, first, an increase in intracranial pressure, and second, an increased venous pressure.

But as a further control and in order more fully, if possible, to establish the fact that the foregoing factors are not capable of singly causing the formation of an optic disk edema, the following experiment was performed.

Two male fox terrier dogs of as near as possible the same weight were subjected to the same intracranial pressure for a period of four hours. A common pressure flask was used for the two dogs. One rubber tube led from the bottle to within 12 inches of the dogs' heads. Here a "T" tube was inserted and connections were made with the cannulas by two equally long pieces of pressure tubing. Tracing of the arterial and venous pressures were not

taken in order to obviate the necessity of possibly vitiating the results from trauma to the animals. These animals are Dogs 10 and 11. Dog 10 was simply anesthetized with ether while his partner, Dog 11, received 5 c.c. of a 0.1 per cent. solution of atropin hypodermically before the ether anesthesia was begun. Table 3 is a record of the observations made on both of these animals.

No further eye changes developed within the next hour and a half in Dog 10. Dog 11 began to show an increased tortuosity of the retinal veins at 5:15 p. m., but by 5:30 p. m., when both dogs were killed, no evidence of optic disk edema or of exophthalmos was present in this animal.

The difference in the effect of this long continued high intracranial pressure on the atropin-free and on the atropinized dog is illustrated even better in the accompanying Figure 12; A, represents the degree of choked disk developed in the right eye of Dog 10, and B, is from the right eye of Dog 11. Both A and B show a marked dilatation of the optic nerve sheath. This was much more pronounced when the tissues were fresh and has decreased as a result of shrinkage during fixation and in the process of making the sections. In the illustration from the eye of Dog 10 there is shown a pronounced choked disk, which contrasts sharply with that from Dog 11, in which the disk is practically normal. A slight deviation from the normal consists here in the well filled retinal veins. As has been stated, however, this congestion occurred during the last ten minutes just previous to the animal's death, when the heart action suddenly weakened, along with respiratory failure. Microscopic examination of the sections from the eyes of Dog 10 shows that the optic nerve fibers are separated and that a finely granular substance fills the interstices, duplicating in all respects the picture obtained in the choked disk that developed in response to the presence of a hydrocephalus from blocking the aqueduct of Sylvius. No such findings are present in the sections from Dog 11.

Consideration of the experimental evidence gathered in this work leads to the conclusion that the absence of a high venous pressure in the presence of an increased intracranial pressure is sufficient to prevent the formation of a choked disk. Logically, then, the next question must be, Can a venous stasis alone cause an optic disk edema?

This question has had to be answered in the negative by various investigators.

The work of Uhthoff and of Cushing and Bordley needs to be especially mentioned in this connection. In order, however, to be able to observe personally what changes do occur when a rise in venous pressure alone is brought about, both the external jugular veins of a small mongrel dog were ligated. Within a few minutes his facial veins filled up and became prominent. Likewise his conjunctival and

anterior ciliary veins. The ophthalmoscope showed an increased tortuosity present in the retinal veins but no sign of any optic disk edema, even an hour later. A further examination several hours later showed still an increased prominence of the retinal veins, but no choked disk. After twenty-four hours the retinal vessels appeared normal again and no further changes occurred during forty-eight hours, after which time the ligation around both jugulars was removed. The result was entirely negative as far as the production of a choked disk was concerned. The effect, then, of a venous stasis alone is well summarized by Cushing. He says: "Simple venous stasis in the retinal veins fails to produce anything more than the congestive features of venous engorgement which accompanies a choked disk and never leads directly to a definite edema of the papilla."

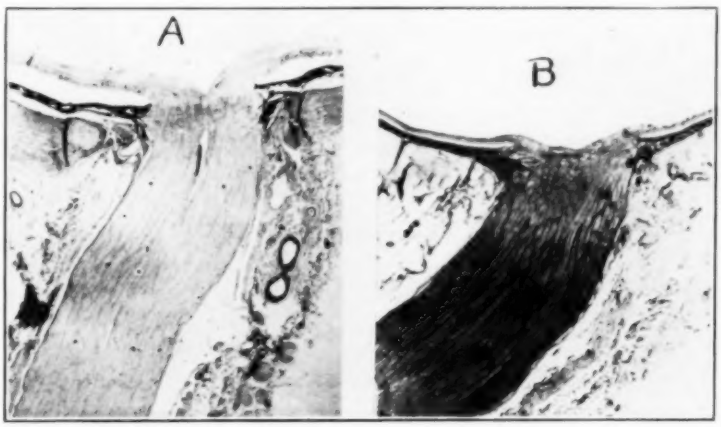

Fig. 12.—Photomicrograph of sections from the right eyes of Dogs 10 and 11, Series II, to show the different effect from the equally increased intracranial pressure in an atropin-free and a heavily atropinized dog.

DISCUSSION

The purpose of the foregoing has been to establish clearly that the origin of choked disk is dependent on a combination of two factors. These are, first, an increased intracranial pressure, and second, an increased venous pressure. This second factor in all probability is due to the presence of the first, but if proper precautions are taken a high venous pressure can be prevented, and with it the formation of an optic disk edema.

Recognition of these facts will readily explain the almost uniform occurrence of choked disk in certain pathologic conditions in man. Thus, in cases of brain tumor or of chronic brain abscess, or, again,

in the hemorrhagic type of pachymeningitis, conditions are present that are almost uniformly accompanied by choked disk. And these cases all show a bradycardia and an increased cerebrospinal fluid pressure. Often the bradycardia presages the advent of the condition. Here often the pulse rate drops to 45 or 50 per minute and with a low arterial pressure such a pulse rate must mean a high venous pressure. These are the types of cases in which both conditions necessary for the formation of a choked disk are present, and, as a matter of common observation, the latter does develop much more frequently than not.

On the other hand, it was early recognized by Gowers that in advanced cases of acute miliary tuberculosis the pulse rate may be extremely low and be accompanied by a low arterial pressure and still these patients never develop more than the congestive features of choked disk. An actual edema never develops. Here the other necessary factor, the increased intracranial pressure, is absent.

Again, the increased intracranial pressure may be present as it undoubtedly is in the so-called condition of "wet-brain" in delirium tremens and still with the accompanying high pulse rate and arterial pressure and failure of development of high venous pressure no choked disk develops.

Optic disk edema develops in man where an increased cerebrospinal fluid pressure and a slow heart rate exist, as can be observed clinically. In these experimental hydrocephalic dogs a condition of high cerebrospinal fluid pressure plus a venous congestion leads to a similar picture. Then, if to a recognition of these two factors that of the lymphatic drainage from the retina, disk and nerve are added, all necessary data for the explanation of the origin of choked disk are at hand. As a result of the engorgement of the retinal veins due to the high venous pressure, an excessive transudate of lymph into the tissues of the retina and disk occurs. Since no proper lymph vessels exist, the tissues distend (Fig. 7) until the pressure exerted in the stretching comes to equal that which obstructs the drainage. The obstruction is the pressure of the cerebrospinal fluid within the sheath lymphatic spaces transmitted from the subarachnoid space of the brain. If this pressure is removed as in ordinary decompression operations the edema in the disk disappears because lymph drainage is again established. As long, however, as the obstructive pressure is greater or maintains a balance with the lymph pressure exerted in the stretching of the retinal tissues and disk, an optic disk edema will continue to exist.

That the increased venous pressure as manifested by an engorgement of the retinal veins cannot be due to a local cause such as direct pressure of the fluid within the optic sheath pressing on the nerve

structure, as was argued by Manz, is definitely proved by these experiments. These show that such retinal venous prominence is simply a local manifestation of a general high venous pressure which manifests itself in all the veins. Whether this high venous pressure is entirely due to the accentuated inhibitory action of the vagus consequent to central stimulation from the high cerebrospinal fluid pressure may be a question open for further investigation. The fact remains, however, that if such inhibitory action is removed by the use of atropin a high intracranial pressure is insufficient to produce a choked disk.

Nor can it any longer be said that the theory of Schmidt-Rimpler is a sufficient explanation. If, according to his suggestion, it were so simple a matter as a direct driving of cerebrospinal fluid through the lamina cribrosa with the consequence of an edematous infiltration of the nerve head, then a choked disk would develop equally well in animals with a normal or with a high venous pressure. But such has not occurred in these experiments. By the use of atropin the venous pressure has been kept normal, and in these cases when an increased intracranial pressure was induced beyond that, even, which in the animals without atropin caused a pronounced choked disk, no optic disk changes occurred.

SUMMARY

To summarize the results of these experiments it may be said:

1. The production of hydrocephalus in dogs is accompanied by a distended optic sheath, hemorrhages and engorgement of the retinal veins and an edematous swelling of the nerve head, thus presenting features which characterize choked disk in man.

2. The introduction of fluid under tension into the subdural space will also produce a like picture.

3. The introduction of fluid under tension into the subdural spaces in a dog that is thoroughly atropinized will lead to nothing further than just the congestive features of choked disk, and this only after a long period of sustained high intracranial pressure.

4. A venous stasis produced by ligation of the external jugular is insufficient to cause any optic disk changes.

5. The production of choked disk in cases of hydrocephalus is not dependent on the presence of inflammatory factors but arises mechanically as a result of a high venous pressure and an obstructed lymph drainage, both caused by the primary factor, the increased intracranial pressure.

THE TREATMENT OF BRONCHIAL ASTHMA WITH VACCINES *

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In a recent article[1] it was shown that 191, or 48 per cent., of patients with bronchial asthma were sensitive to some type of protein. In a more recent article[2] the results of treatment with proteins of 100 of the sensitive patients was given; this 100 included only those patients who were sensitive to the proteins of animal hair and emanations and of food. The present paper concerns those patients who were treated with vaccines and represents a total of 178 individuals. Twenty-eight of these patients were sensitive to bacterial proteins, and therefore these patients were treated with vaccines of the organisms to which they were sensitive. The remaining 150 patients included in this paper were not sensitive to any protein with which they were tested. Since these nonsensitive patients are a part of the whole series of 400 cases, 48 per cent. of whom were found to be sensitive, it may be assumed, as shown in the previous paper, that these nonsensitive patients were tested with a sufficient number of proteins to justify the statement that they belong in the nonsensitive group. Therefore, this paper comprises all of those patients in the series of 400 studied who were sensitive to and treated with bacteria (28 in all), and all of those nonsensitive patients who had a reasonable amount of treatment (150 in all).

Since the aim of these studies,[3] of which this article is a part, has been to determine the cause, and consequently the treatment, of bronchial asthma, the patients represented in this article have been treated in various ways; an outline of the various methods of treatment follows. The first part of the paper concerns those patients who were sensitive to bacterial proteins, and, as already mentioned, these patients were treated with vaccines of the bacteria to which they were

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* From the Medical Clinic of the Peter Bent Brigham Hospital.

1. Walker, I. C.: A Clinical Study of 400 Patients with Bronchial Asthma, Boston M. and S. J. **179**:288, 1918.

2. Walker, I. C.: Treatment of Bronchial Asthma with Proteins, Archives Int. Med. **22**:466, 1918.

3. Studies on bronchial asthma were made possible through a gift by Mr. Charles F. Choate, Jr., of Boston to the Peter Bent Brigham Hospital. A complete list of the papers is given with the article in Arch. Int. Med. **22**:466, 1918.

sensitive. Since the other patients were not sensitive to proteins, they were thought to be of an infectious type and the infection was considered to be in the respiratory tract. Therefore, vaccines were made from the organisms found in the patients' sputum or nasal secretions. Since these sources produced such a wide range of bacteria, it was thought best and simplest first to find out what part those organisms which only grow on plain agar might play in the cause of bronchial asthma; the results of this work are given in the second part of the article. We were next interested in the part those organisms which grow only in dextrose bouillon and not in plain agar might play in the cause of bronchial asthma; thus, the third part of this article deals chiefly with streptococci and pneumococci. It was learned that some patients were relieved of asthma by plain agar vaccines and that others were relieved by bouillon vaccines, but still there were many in each group that were not relieved. Therefore, a series of patients were treated in both ways; that is, some who were not relieved by vaccines made from growing the sputum on plain agar were then treated with vaccines made by growing the sputum in dextrose bouillon, and vice versa; also blood agar plates were usually made from a shaken emulsion of the sputum in dextrose bouillon in order to determine the predominating organism and to identify the various types of bacteria. Thus the fourth part of the paper deals with the treatment by vaccines which were made both ways. In other papers we have already called attention to the fact that the nonsensitive as well as the sensitive patients with bronchial asthma may have asthmatic attacks throughout the year or only at various times or seasons of the year. It is obvious that it is impossible to prognosticate results from vaccine treatment in patients who have asthma only for a period of a month or six weeks once or twice a year, as is the case with some patients who have an early spring attack or a late fall attack or two such attacks; therefore, patients who have such limited asthma are not included at all in this paper, although there is evidence that bacteria are frequently the cause of such asthma. The method of treatment which has already been outlined in this paper concerns only those patients who have either continuous or frequently repeated attacks of asthma throughout the year, or those who have asthma from early fall throughout the winter and early spring; that is to say, they have asthma throughout the greater part of the year. Even in these latter cases one must be very cautious in interpreting benefit from treatment since a mild winter or an early spring might account for the improvement in the asthma, and for this reason we have attempted to be very conservative in our results. There is, however, still another type of asthma to which attention should be called since it is an entity and closely

simulates asthma caused by pollens. This is a nonsensitive type of summer asthma caused by bacteria, and it is usually confined to the months of late May, June, July and early August, therefore closely simulating the early pollen asthma. A small series of this summer nonsenstive type of asthma will be presented in this article. Finally, attention will be called to specificity among bacteria and proteins in the treatment of bronchial asthma, and proof of such specificity, including the use of intravenous typhoid vaccine and subcutaneous peptone, will be given.

Patients sensitive to bacterial protein and treated with vaccines.—As has been said, twenty-eight patients were sensitive to and treated with bacteria. In addition to these there were eleven other patients who were sensitive to bacterial proteins, but as they were also sensitive to other proteins, these patients were included in the preceding article,[2] and therefore will not be discussed here. Two other patients are not discussed here because they were not seen after their first visit. Therefore, in the total of 400 patients on which these studies have been based, forty-one, or 10 per cent., were sensitive to bacterial proteins. Two different standards must be considered when reading skin tests with bacterial proteins. One type of positive reaction is an urticarial wheal measuring at least 0.25 to 0.5 cm. in diameter, which appears within one-half hour, and the other type is a delayed reaction which occurs hours later, and the next day the site of the inoculation is inflamed, hot, and elevated, and resembles an infection.

The treatment of the twenty-eight patients who were sensitive to and treated with bacteria consisted of either stock or autogenous vaccines of those bacteria to which they were sensitive. Treatment was given with the whole killed bacteria in the form of a vaccine, because by so doing the patient would be apt both to acquire an immunity to the bacteria and become desensitized to the protein of the bacteria, whereas treatment with the protein of the bacteria alone would only desensitize and not immunize; in other words, treatment with the protein alone simply protects the patients against bacterial protein, but as the bacteria are constantly present, the infection may at any time become so great as to undo suddenly what has been accomplished in the way of desensitization. A parallel instance is that of attempting to desensitize pollen cases while the patients are being exposed to the pollen, or of attempting to desensitize a wheat case while the patient is eating wheat. Treatment with vaccines of the bacteria to which the patient is sensitive both immunizes and desensitizes at the same time; thus protection against living bacteria parallels desensitization. It seemed to make no difference whether the vaccines

were stock or autogenous. A detailed discussion of the skin test and treatment with bacteria was presented in Studies III[4] and XIV.[5]

A discussion of the twenty-eight patients who were sensitive to and treated with bacteria follows. Seventeen patients were sensitive to and treated with *Staphylococcus pyogenes aureus;* ten were relieved of asthma, six were markedly improved and one was not benefited at all. One patient was sensitive to and relieved by treatment with both *S. pyogenes aureus* and *albus*. Two patients were sensitive to both *S. pyogenes aureus* and *Streptococcus hemolysans,* and both were relieved by treatment with *S. hemolysans;* one was markedly improved by *S. pyogenes aureus* before treatment was begun with *S. hemolysans* and the other was not treated with *S. pyogenes aureus*. One patient who was sensitive to both *S. pyogenes aureus* and diphtheroid was markedly improved by *S. pyogenes aureus* vaccine and was later relieved of asthma by diphtheroid vaccines. Two patients who were sensitive to only *S. pyogenes albus* were relieved by treatment with this organism. One patient who was sensitive to both *S. pyogenes albus* and *S. pyogenes,* was markedly improved by *S. pyogenes albus* vaccine and was later relieved of asthma by *S. pyogenes* vaccine. Three patients were sensitive to the protein of *S. hemolysans* alone, and treatment with vaccine of this organism relieved the asthma. The remaining patient in this group of twenty-eight was sensitive to both *S. hemolysans* and *S. viridans,* and treatment with vaccines of both organisms relieved the asthma. Therefore, among the twenty-eight patients who were sensitive to and treated with bacteria, there was only one failure and six others who were not relieved but were markedly improved; these seven were sensitive to and treated with *S. pyogenes aureus*. There were no failures among those who were sensitive to and treated with the other bacteria, probably because they were not so frequent. In other words, 75 per cent. of the patients who were sensitive to and treated with bacterial proteins were relieved of asthma; it is interesting that these results are the same as were obtained by the treatment of patients with other proteins to which they were sensitive. (See previous article.)

The age of onset and the duration of asthma in the twenty-eight patients who were sensitive to and treated with bacteria follows: Three patients began to have asthma between the ages of 1 and 5; two of these who had had asthma for 5 and 20 years, respectively, were relieved, and the other one who was not benefited had had asthma for 7 years. Three began to have asthma between the ages of 5 and 10; all three were relieved and the duration of asthma in each case

4. Study III, Jour. Med. Research **35**:487, 1917.
5. Study XIV, Jour. Med. Research **36**:423, 1917.

was 2, 10 and 12 years. Five patients began to have asthma between the ages of 10 and 20; one who had had asthma for only one year was markedly improved, and the other four who had had asthma for 9, 13, 19 and 25 years, respectively, were relieved. Five patients began asthma between the ages of 20 and 30; all of these were relieved and the duration of asthma in these was 1, 4, 5, 5 and 10 years, respectively. Ten patients had their first attack between the ages of 30 and 40; the four who were markedly improved had had asthma for 1, 2, 3 and 14 years, whereas the other six patients who were relieved had had asthma for 1, 1, 2, 9, 10 and 30 years, respectively. Only two patients in this group began to have asthma after 40; one who had the onset at 42, with a duration of 3 years, was relieved, whereas the other who began to have asthma at 52, with a duration of one year, was markedly improved. Therefore, the age of onset had no bearing on the benefit from treatment, as many of those beginning to have asthma during adult life were relieved as of those who began to have asthma during childhood. Neither in these cases did the length of time which the patients had had asthma influence treatment; in fact, those who were relieved had had asthma for much longer periods than had those who were not relieved.

On comparing this group of sensitive cases with the sensitive ones presented in the preceding article it is noted that during childhood sensitization to the bacterial proteins corresponds relatively closely to those sensitive to other types of protein, but that during adult life sensitization to the bacterial proteins is relatively much more frequent than it was for other types of proteins. The duration of asthma among patients who were sensitive to the various types of proteins was relatively about the same and the percentage of cases being relieved was exactly the same for all proteins. The permanency of relief among those who were sensitive to and treated with bacteria is of much shorter duration than for those who were sensitive to and treated with animal hair proteins. With the bacterial cases relief continued for only a few months after treatment was stopped, and naturally this is all that would be expected for such a short course of treatment, together with the constant exposure to bacteria. Relief continues, however, as long as treatment is given, and in this way the bacterial cases resemble the food cases, since the food cases are free from asthma as long as those foods to which the patient is sensitive are omitted from the diet.

Nonsensitive patients treated with plain agar organisms.—Seventy-five patients were treated with vaccines made from the bacteria which were recovered from the patient's thick sputum when the sputum was streaked on large surfaces of plain agar. The various types of organisms were identified after twenty-four hours' growth, and the pre-

dominating organism was usually made into a vaccine and given to the patient, although in some instances a stock vaccine of that particular type of organism was given instead of the autogenous vaccine, and in a few instances a combination of sputum organisms was given. Thirty-five, or 46.6 per cent., of these patients were relieved of asthma, twelve others, or 16 per cent., were greatly improved, and twenty-eight, or 37.3 per cent., were not benefited.

The types of organisms recovered from the sputum and incorporated into vaccines in these 74 patients were as follows. Of the 35 patients who were relieved of asthma, in 8 cases the vaccine comprised *S. pyogenes aureus* alone, in one case a mixture of *S. pyogenes aureus* and *albus*, in one case a mixture of *S. pyogenes aureus* and a gram-negative staining bacillus,[6] in one case a mixture of *S. pyogenes aureus* and a diphtheroid organism, in one case *S. pyogenes albus* alone, in 17 cases a diphtheroid organism alone, in 3 cases a gram-negative staining bacillus[6] alone, in one case Friedländer's bacillus alone, and in the remaining 2 cases the organism was not identified. One of the foregoing patients who was relieved by diphtheroid vaccine also had Friedländer's bacillus in large numbers, but vaccines of this organism did not improve the patient, and another of the foregoing patients who was relieved by diphtheroid vaccine was not benefited by *S. pyogenes aureus* which was also present in the sputum in large numbers; therefore, these instances show the specificity of bacteria in the treatment of asthma. Of the 12 patients who were markedly improved, 7 were treated with diphtheroid vaccines alone, 2 with a mixture of *S. pyogenes aureus* and diphtheroid, 2 with *S. pyogenes aureus* alone and 1 with the gram-negative staining bacillus. One of those who was markedly improved by diphtheroid vaccine was not benefited by *S. pyogenes aureus* vaccine, and another who was markedly improved by diphtheroid vaccine was not benefited by the gram-negative staining bacillus, thus again showing the specificity of bacteria in the treatment of asthma. The 28 patients who were not benefited were given vaccines as follows: 8 were given *S. pyogenes aureus* alone, one *S. pyogenes aureus* alone and later *S. pyogenes albus* alone, 4 were given *S. pyogenes aureus* alone and later diphtheroid alone, one a combination of *S. pyogenes albus* and diphtheroid, one *S. pyogenes albus* alone, and the remaining 13 cases were given vaccines of diphtheroid organisms alone. A summary of the results from the use of vaccines made from the several types of predominating organisms in the sputum when grown on plain agar was as follows: diphtheroid alone,

6. This gram-negative staining bacillus, which will be frequently mentioned, was more or less motile, sometimes only sluggishly motile, at other times very actively motile; in the litmus-sugar-serum waters it reacted like the colon type of bacillus although it did not produce indol in Dunham's peptone solution.

17 relieved, 7 improved and 13 not benefited; *S. pyogenes aureus* alone, 8 relieved, 2 improved and 8 not benefited; *S. pyogenes albus* alone, 1 relieved and 1 not benefited; gram-negative staining bacillus, 3 relieved, 1 improved and 1 not benefited; Friedländer's bacillus, 1 relieved and 1 not benefited; since the remaining patients received a combination of bacteria in the vaccines, results cannot be attributed to any special type of organism.

The relationship between the age of onset of asthma and the results from vaccine treatment in this series of cases follows: Of those who began to have asthma between the ages of 1 and 5 years, 12 were relieved, 1 was improved and 2 were not benefited; of those whose onset of asthma began between the ages of 5 and 10 years, 5 were relieved and 2 were not benefited; of those whose onset began between the ages of 10 and 20, 3 were relieved, 1 was improved and 3 were not benefited; onset between 20 and 30, 3 were relieved, 7 were improved and 3 were not benefited; onset between 30 and 40, 9 were relieved, 3 were improved and 7 were not benefited; onset between 40 and 50, 2 were relieved and 7 were not benefited; onset above 50, none was relieved, 8 were improved and 4 were not benefited. It is evident that three-fourths of those who began to have asthma in childhood (from 1 to 10 years) were relieved; that half of those whose onset was between the ages of 10 and 40 were relieved; that a third of those whose onset was between 40 and 50 were relieved, and that after 50 none was relieved. Therefore, in general, the younger the patient is when asthma begins the better the prognosis, and in children the prognosis is very good.

Before we prognosticate, however, we must see what effect the duration of asthma may have on the results of treatment. Of those who had had asthma for one year, 3 were relieved, 2 improved and 4 not benefited; duration for two years, none relieved, 2 improved and 3 not benefited; three years' duration, 4 relieved, 2 improved and 4 not benefited; duration four years, 3 relieved, 1 improved and 1 not benefited; five years' duration, 2 relieved and no failures; six years' duration, 3 relieved and 1 not benefited; seven years' duration, 1 relieved and 2 not benefited; eight and nine years' duration, 1 each relieved and no failures; ten, twelve and fourteen years' duration, 2, 2 and 3, respectively, relieved and 1, 2 and 2, respectively, not benefited; sixteen years' duration, 2 relieved and 3 not benefited; twenty to thirty years' duration, 8 relieved and 5 not benefited. In other words, of those whose duration was from 1 to 5 years, 12 were relieved and 12 were not benefited; duration from 5 to 10 years, 8 were relieved and 4 were not benefited; duration from 10 to 20 years, 7 were relieved and 9 were not benefited; duration from 20 to 30 years, 8 relieved and

3 not benefited. Therefore, in these cases the length of time that a patient has had asthma makes little difference in the prognosis, and the duration in years of those relieved so closely parallels the duration of those not benefited that the latter serve as a good negative control for the positive results in the former.

From what has been shown in these cases it is evident that the best prognosis concerns those patients who begin to have asthma early in life and have not yet reached the age of 40 when treatment is begun, and that the worst prognosis concerns those patients who begin to have asthma late in life no matter for how short a time they have had it. In other words, the age of the patient when treatment is begun is an important fact, and the older the patient when treatment is begun the worse the prognosis. This is naturally what one would expect, because the older the patient the more liable is he to be in poor physical condition, and the poorer the patient's condition the less able is he to manufacture antibodies. Vaccines of course stimulate the production of antibodies and therefore they fail if the patient is unable to respond to their stimulus. The duration of relief from asthma in these cases also depends on the patient's ability to manufacture antibodies, and in this sense we mean resistance to disease. Some patients will remain free from asthma for months after treatment is discontinued, whereas others require the constant use of vaccines in order to be free, and when vaccines are stopped the condition quickly returns. Patients who have paroxysmal attacks of asthma, no matter how short the interval of freedom between attacks, are much easier to relieve of asthma than are those patients who have continuous asthma with no free intervals. Here again the principle of resistance enters and the fact that a patient has at times freedom from asthma means that he does have more or less resistance to the bacteria causing the trouble, and the fact that some patients have continuous asthma means that those patients never develop enough antibodies or resistance to at times temporarily recover from asthma. Therefore the prognosis in the nonsensitive asthmatic patient depends equally on the patient's resistance and on the selection of the proper vaccine for treatment.

Nonsensitive patients treated with organisms grown in dextrose bouillon.—This group of cases includes only those patients whose sputum when inoculated into dextrose bouillon grew types of organisms which resembled a streptococcus morphologically, and which, in addition, were gram-positive staining and bile insoluble. Therefore, in these cases the predominating organism when the sputum was grown in dextrose bouillon was probably a streptococcus, although no attempt was made in these instances to identify the particular type of

streptococcus, and vaccines were made directly from the dextrose bouillon culture after from twenty to twenty-four hours' growth by centrifugalizing out the bacteria, washing in normal saline and then suspending the washed bacteria in normal saline.

A total of twenty-four patients were treated with vaccines made in this manner. Nine, or 37.5 per cent., were relieved of asthma; six, or 25 per cent., were markedly improved, and nine, or 37.5 per cent., were not benefited at all.

The preceding groups of cases were discussed in considerable detail in regard to the age of onset and duration of asthma. This last group of cases is so small in number that much space and time may be saved by the use of a table from which general conclusions may be drawn at a glance (Table 1).

TABLE 1.—Results of Treatment of Nonsensitive Patients with Dextrose Bouillon Cultures

Relieved of Asthma			Markedly Improved			Not Benefited		
Number of Patients	Age of Onset, Years	Duration of Asthma, Years	Number of Patients	Age of Onset, Years	Duration of Asthma, Years	Number of Patients	Age of Onset, Years	Duration of Asthma, Years
1	1	20	1	1	25	1	4	60
1	3	2				1	8	21
1	5	1	1	5	26	1	21	12
1	7	30				1	26	18
1	10	16				1	28	18
1	25	1	1	22	5	1	30	20
1	40	4	1	32	10	1	36	3
1	41	1	1	42	5	1	37	2
1	29	20	1	37	15	1	40	2
9 or 37.5%			6 or 25%			9 or 37.5%		

Although the relationship in this group of cases as regards the age of onset and the duration of asthma between those who were relieved, improved and not benefited is too close to warrant definite conclusions, still the evidence is in favor of what the previous group of cases conclusively showed. Namely, that the younger the patient is when asthma began, irrespective of the duration, the better the prognosis, and that the older the patient is when asthma begins, no matter how short a time he has had asthma, the poorer the prognosis. The permanency of relief in the patients treated with streptococcus vaccines does not seem to differ from that in those treated as previously outlined in this paper.

Nonsensitive patients treated with several different vaccines.—The patients in this group were not only treated with the predominating

organism in their sputum when it was grown on plain agar and in dextrose bouillon, but also, if the patient was not relieved by such vaccines, other types of organisms were employed. In other words, different types of vaccines were tried, each one for a period of several weeks, until the proper one was found and relief or marked improvement resulted. Since, as already shown in this paper, of the organisms which were recovered from plain agar, the diphtheroid and *S. pyogenes aureus* usually predominated and were found to give relief most frequently, and of the organisms which were recovered from dextrose bouillon, the streptococci usually predominated and were found to give relief most frequently, these were the first to be tried and consequently they were used in most instances. When these failed, other organisms were used, so that in the cases which were not relieved at all, many different types of vaccines were employed. This method of treatment in these cases does not show the relative merits of growing bacteria for vaccines on any special kind of mediums, but it does show clearly the specificity of bacteria in the treatment of bronchial asthma.

Thirty-five patients were treated as outlined in the foregoing. Eleven, or 31.4 per cent., were relieved; 8, or 23 per cent., were markedly improved, and 16, or 45.6 per cent., were not benefited at all.

Of the eleven patients who were relieved, one was relieved by diphtheroid vaccines after streptococci had failed, one was relieved by a mixture of *S. pyogenes aureus* and *albus* after streptococci had failed; four were relieved by streptococci after diphtheroid vaccines had failed, and in one of these *S. pyogenes aureus* had also failed; the remaining five patients were relieved by *S. hemolysans* vaccines after diphtheroid vaccines had failed, and in three of these *S. pyogenes aureus* vaccine had also failed.

Of the eight patients who were markedly improved, two were markedly improved by diphtheroid vaccines after streptococci and *S. pyogenes aureus* vaccines had failed; one was markedly improved by streptococcus vaccines after diphtheroid vaccines had failed, and the remaining five patients were markedly improved by *S. hemolysans* vaccines after diphtheroid vaccines had failed, and in three of these *S. pyogenes aureus* vaccines and in another *S. albus* vaccine had also failed.

The sixteen patients who were not benefited were treated with the following types of vaccines: 13 with diphtheroids, 12 with streptococci, 4 with *S. hemolysans*, 7 with *S. pyogenes aureus*, 3 with *S. pyogenes albus*, 2 with gram-negative staining bacillus, and 1 with *B. pyocyaneus*, which happened to be present in the sputum in pure culture.

Therefore, in this group of cases the treatment of the patients who were relieved and who were markedly improved shows the specificity of bacteria in the treatment of bronchial asthma in that only one of many kinds of vaccines gave good results. The failure in treatment in the sixteen cases, although a wide range of bacteria were used in the vaccines, shows that nonspecific vaccine treatment is not beneficial in bronchial asthma.

Table 2 gives the age of onset and the duration of asthma in the thirty-five patients who were treated with many types of vaccines.

TABLE 2.—RESULTS OF TREATMENT WITH VARIOUS TYPES OF VACCINES

Relieved of Asthma			Markedly Improved			Not Benefited		
Number of Patients	Age of Onset, Years	Duration of Asthma, Years	Number of Patients	Age of Onset, Years	Duration of Asthma, Years	Number of Patients	Age of Onset, Years	Duration of Asthma, Years
1	1	11				1	5	25
1	2	4				1	6	9
1	7	2	1	6	37	1	6	55
1	8	13	1	8	30	1	7	9
1	13	37	1	10	40	1	16	4
1	18	20				1	16	15
1	19	24				1	31	4
1	20	22	1	21	8	1	34	2
1	24	14	1	25	3	1	35	3
1	26	1	1	36	2	1	35	13
1	34	5	1	50	5	1	35	17
			1	55	9	1	36	6
						1	40	6
						1	57	2
						1	60	1

In Table 2 it is noted that although the duration of asthma in the cases not benefited averages considerably less than the duration in the relieved cases, the age of onset of asthma, and consequently the age of the patient when treated, averaged much greater in the not benefited patients than in the relieved cases. Therefore, the large percentage of failures in the vaccine treatment in this group of cases, in spite of the fact that a wide range of vaccines including the predominating organism as well as others were used, was probably due to the more advanced age of the patient both when asthma began and when treatment was instigated.

Nonsensitive summer asthmatics treated with vaccines.—Since it is generally believed that the type of asthma which is limited to the

summer months is caused by pollens, it seems advisable to discuss briefly the following sixteen patients who were not sensitive to pollens although their attacks of asthma closely paralleled the pollination of plants. Of five patients who had had asthma all summer for five or more summers, with no asthma at other times of the year, and who would be considered sensitive to both the early and the late pollens, although they were not sensitive at all to pollens, two were relieved of asthma by vaccines of streptococci which predominated in their sputum when cultured in dextrose bouillon. One of these patients was not benefited by vaccines made from growing the sputum on plain agar, although she was relieved for two successive summers by streptococcus vaccines. A third patient was relieved by vaccines of *S. pyogenes albus* for two successive summers; this organism was used in the vaccine because it was present in the patient's nasal secretion in pure culture, and his nasal secretion was troublesome. The other two patients were not benefited by vaccines consisting of *S. pyogenes aureus, S. pyogenes albus* or diphtheroids. Two patients who had asthma only during June and July, the early pollen season, were not sensitive to pollens. One patient was relieved by vaccines of *S. pyogenes aureus* and the other patient was relieved for two successive summers by vaccines of *S. pyogenes albus;* the latter patient had had asthma for sixteen successive seasons. Three patients who had asthma only during July and August were not treated, because of the short duration of their attacks, and, furthermore, these untreated patients served as controls for the treated patients. Five patients had asthma during June, July and August. One patient was improved one summer by diphtheroid vaccines and was relieved the next summer by streptococcus vaccines; another patient was markedly improved by streptococcus vaccines; another patient was not benefited one summer by vaccines made from growing the sputum on plain agar, but was markedly improved the next summer by *S. hemolysans* vaccine; another patient was somewhat benefited one year by plain agar vaccines and another year by dextrose bouillon vaccines, but neither type of vaccines gave marked relief; the fifth patient was not benefited at all by streptococcus vaccine. Therefore, of sixteen nonesensitive summer asthmatics who were given vaccines, five were relieved of asthma, four were markedly improved, four were not benefited and three were not treated.

The following comparisons as regards treatment may be made between sensitive and nonsensitive asthmatic patients. In the previous article, which concerned patients sensitive to proteins, and in the first part of this article which concerns patients sensitive to bacterial proteins, it was shown that in general a favorable prognosis could be

anticipated irrespective of the age of onset of asthma or the age of the patient when treated. With the nonsensitive patients, however, the later the age of onset and the later the age of the patient when treatment is begun, the more unfavorable the prognosis. The duration of asthma alone played little part in the prognosis in either type of case. Seventy-five per cent. of the sensitive patients were relieved of asthma by treatment with the proteins to which they were sensitive, whereas only 40 per cent. of the nonsensitive patients were relieved of asthma by treatment with vaccines. The permanency of relief from asthma in the sensitive patients was of much longer duration than in the nonsensitive patients. Both the sensitive and the nonsensitive patients illustrate specificity in the treatment of bronchial asthma; that is, the specificity of proteins in the treatment of sensitive cases and the specificity of bacteria in the treatment of nonsensitive cases. We have, however, only inferred that nonsensitive asthmatic patients are not benefited by treatment with proteins. Because of the more or less general belief that infections may be alleviated by nonspecific protein therapy — and in the case of chronic arthritis this is frequently found to be true — it seems worth while to mention our results in the treatment of the infectious or nonsensitive type of bronchial asthma with proteins.

Many of the nonsensitive or infectious type of asthmatics have been treated with various proteins. Three patients who were in the hospital wards because they were having severe asthma every day were given, intravenously, typhoid vaccine without improvement in the asthmatic symptoms. A week later a larger dose was given without any benefit. After this the patients were given, subcutaneously, two hundred million autogenous streptococcus vaccine made from growing their sputum in dextrose bouillon. A few days later one patient was somewhat improved, another seemed a little better and the third was not improved. A week later still the autogenous vaccine was increased to 250 million and a few days after this one patient was very much better, another was considerably improved and the third was somewhat better. The autogenous vaccine was given each week with gradual improvement in each instance until two patients left the hospital three weeks later and the third patient was able to leave in five weeks. Therefore, the intravenous typhoid vaccine was followed by no benefit, whereas the autogenous streptococcus vaccine was followed by a gradual though distinct improvement. Several of the nonsensitive summer asthmatics and some of the other non-sensitive cases were given courses of treatment with various pollens without benefit. A few nonsensitive patients were given wheat proteins and a few were given large doses of peptone subcutaneously without benefit. This

latter method of treatment is dangerous unless the patient is tested with peptone to rule out the possibility of his being sensitive to it. We feel that this fad of injecting patients with proteins to which they are not already sensitive is, in general, apt to be a mistake; the possibility of sensitizing patients to proteins, exclusive of typhoid vaccine, seems to outweigh the chance of improvement by such treatment.

In this work we have made our vaccines in different ways in the hope of making them more efficient. At first toluol was used as a preservative, but because of frequent, small, sore indurations at the site of inoculation, toluol was given up and 0.25 per cent. phenol was substituted; the phenol has been very satisfactory. For a long time we killed our vaccine by heating at 56 C. for two hours; lately, however, we have heated it at 56 C. for only three quarters of an hour and we are inclined to think better results follow the shorter heating. A leukocytic extract was also given with the vaccines without any beneficial effect; we have not given sensitized vaccine. Patients react differently to different amounts of vaccine; some patients will improve on small gradually increased amounts, whereas other patients do not improve until large amounts are given. Therefore, we now make two dilutions of each vaccine, one containing 1,000 million to the cubic centimeter, and the other containing 10,000 million to the cubic centimeter. The weaker vaccine is given first for several doses in rapidly increasing amounts in order to see how the patient reacts, and if neither beneficial nor unfavorable results follow, then the stronger vaccine is used, but if desirable results follow the weaker vaccine this is then given in gradually increased amounts at weekly intervals; the first dose is usually about 200 million. In the treatment of bronchial asthma we have given 3,700 doses of vaccine; that is to say, we have taken the trouble actually to count this number of injections and we do not know how many more we have given which we have not attempted to count. In only one instance did a really bad reaction result; that one patient had fever, general malaise and was in bed for two weeks; the vaccine proved to be sterile and the patient had no local reaction, therefore the patient was considered too susceptible for further treatment. Of the 3,700 doses of vaccine given, 800 were *S. pyogenes aureus*, 1,200 diphtheroids, 1,000 streptococcus and the remaining were miscellaneous vaccines.

CONCLUSIONS

Twenty-eight patients with bronchial asthma were treated with vaccines of the bacteria to which they were sensitive; 75 per cent. were relieved of asthma and 21 per cent. were improved.

Seventy-five nonsensitive patients were treated with vaccines made from culturing their sputum on plain agar; the predominating organism was usually the one selected for treatment; 46.6 per cent. were relieved of asthma and 16 per cent. were improved.

Twenty-four nonsensitive patients were treated with vaccines made from culturing their sputum in dextrose bouillon and using only the streptococci; 37.5 per cent. were relieved of asthma and 25 per cent. were improved.

Thirty-five nonsensitive patients were treated with vaccines made from culturing the sputum both ways; in other words, many types of vaccines were used; 31.4 per cent. were relieved and 23 per cent. were improved.

Sixteen nonsensitive summer asthmatics were treated with vaccines; 31.2 per cent. were relieved and 25 per cent. were improved.

Therefore, of 150 nonsensitive asthmatics who were treated with vaccines 40 per cent. were relieved and 20 per cent. were improved; these results should be compared with the treatment of sensitive patients as reported in the previous article, 75 per cent. of whom were relieved, and in this article 75 per cent. of whom were relieved.

With the sensitive cases, the age of onset of asthma, the duration of asthma and the age of the patient when treated had little to do with the prognosis; however, with the nonsensitive cases these facts had much bearing on the prognosis; the older the patient is when asthma begins and the older he is when treatment is begun, the more unfavorable the prognosis from vaccines in nonsensitive cases.

The permanency of relief from vaccines in the nonsensitive cases depends on the individual's resistance to the bacteria in question; therefore, the duration of relief from asthma varies. Some patients continue free from asthma after vaccines are discontinued for many months, others for only a month or two, and some patients require the constant use of vaccines to be free from asthma. Succeeding courses of vaccine treatment, provided that there has been no change in the bacteria which are causing the relapse, seem to relieve more promptly than the first course of vaccine treatment; when a relapse is not relieved by a second course of vaccines which previously did relieve, other bacteria should be suspected as the cause of asthma and new vaccines should be made.

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DIFFERENTIATION OF NEPHROPATHIES, CARDIOPATHIES AND ALLIED CONDITIONS BY THE DETERMINATION OF PHYSICAL CONSTANTS *

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As a means of differentiating nephropathies, cardiopathies, arteriosclerosis and essential hypertension, Butterfield, Erdwurm and Braddock[1] have employed certain physical methods of examining the blood serum, and they claim that each of these conditions is accompanied by characteristic changes that are of diagnostic value. During the two years which have elapsed since the publication of their paper, however, this method of examination has received little attention from clinical laboratories and workers. In order to secure data on the value of the method, we have applied it to a large number of serums, and the results of the investigation are incorporated in the present communication.

The following determinations were made: Refractive index, freezing point, specific gravity, total solids, inorganic salts (ash).

Method of Obtaining Serum.—Blood was drawn between 10 and 12 a. m., the patient having had nothing but a hospital breakfast. It was allowed to clot. Serum was drawn off and centrifuged the same day between 4 and 5 p. m. It was kept in the ice box overnight and the determinations were made the next day.

Methods.—The *refractive index* was determined with the Abbé refractometer. It is expressed as ($\triangle$ Nd $\times 10^5$) and represents the difference between the refractive indexes of serum and the index of water at the same temperature, 25 C., multiplied by 1,000.

The *specific gravity* was determined with the aid of a small (5 c.c.) flask provided with a ground glass stopper having a capillary bore. The weights of both the freshly boiled, distilled water and the serum were obtained in the same way. The flask was filled with the liquid at about 20 C., stoppered, and the excess forced out through the capillary bore. The flask and contents were allowed to warm up slowly to 25 C., then wiped clean with filter paper to remove any of the overflow which may have adhered to the outside, and carefully weighed. From this weight was subtracted the weight of the empty flask. The result gave the weight of the serum in one case and the weight of the same volume of water in the other. Dividing the weight of the serum by that of the water gives the specific gravity.

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1. Butterfield, Erdwurm and Braddock: Am. Jour. Med. Sc., 1916, **151,** No. 63.

The *freezing point* was determined with the Beckmann apparatus in the usual manner. The determinations were made in duplicate. When discrepancies occurred, the determination was repeated three or four times until the right value was obtained. The values represent the fraction of a degree below zero at which the serum freezes.

Total solids were determined in the usual manner by drying to constant weight at a temperature of 90 to 95 C.

Ash was determined by drying, then by ashing at a low red heat so as to avoid the volatilization of the chlorids. The crucibles used were small, weighing between 6 and 8 gm., thus minimizing the effect of "dead" weight.

TABLE 1.—NORMALS

Case No.	Age	Sex	Refractive Index	Specific Gravity	Freezing Point	Solids, per Cent.	Ash, per Cent.
106	26	♂	15.0	1.0263	0.600	8.4	0.75
71	25	♂	15.7	.....	.....	8.8	0.76
72	24	♀	15.7	1.0286	0.612	9.2	0.75
N1	26	♂	15.8	1.0258	0.568		
N2	30	♀	16.2	1.0284	0.562		
N3	45	♀	16.3	1.0283	0.550		
N4	17	♂	16.4	1.0295	0.570		
N5	21	♂	16.5	1.0265	0.570		
104	25	♂	16.5	1.0277	.....	9.4	0.80
N6	45	♂	16.6	1.0295	0.605		
N7	22	♂	16.8	1.0278	0.570		
N8	21	♂	16.9	1.0275	0.576		
107	27	♀	17.0	1.0284	0.595	9.7	0.76
N9	23	♂	17.3	1.0278	0.558		
N10	21	♂	17.4	1.0278	0.567		
N11	20	♂	17.5	1.0292	0.557		
N12	25	♂	17.5	1.0280	0.564		
105	25	♂	17.5	1.0297	0.620	9.9	0.76
N13	20	♂	17.6	1.0297	0.561		
N14	20	♂	17.8	1.0292	0.575		
N15	20	♂	18.0	1.0294	0.595		
N16	28	♂	18.2	1.0298	0.565		
N17	30	♂	18.4	1.0301	0.577		

Grouping of Cases.—It is difficult to find pure cases, that is cases having only one affliction. Therefore, we grouped them according to the most prominent and apparently primary lesion. Whenever the secondary lesion was at all prominent and when it was suspected of modifying the constants due to the primary, it was noted. This was done to throw light on those cases that seemed out of place in the group assigned them by the diagnosis of the attending physicians.

The accompanying tables show the results obtained. The urine picture, the blood pressure and the degree of nitrogenous retention are also given.

TABLE 2.—Chronic Nephritis with Edema

Case No.	Age	Sex	Urine	Blood Pressure	Nitrogen Retention	Condition	Specific Gravity	Refractive Index	Freezing Point	Solids, per Cent.	Ash, per Cent.
27	35	♂	Alb. ++++; many casts	165/100	Slight	Unimproved	1.0198	10.3	0.602	6.1	0.74
26	14	♀	Alb. 7%; many casts	150/100	Slight	Improved	1.0179	10.5	0.761	5.9	0.73
1	14	♀	Alb. 7%; many casts	150/100	Slight	Improved	1.0179	10.5	0.616	5.5	
69	14	♀	Alb. 7%; many casts	150/100	Slight	Improved	1.0180	11.0	0.625	6.5	0.76
25	57	♂	Alb. +; few casts; Exer. 50%	180/92	Slight	Improved	1.0217	11.9	0.672	7.1	0.75
15	42	♂	Alb. ++++; many casts; Exer. 10%	196/99	Slight	Improved	1.0218	12.0	0.671	7.22	0.94
76	38	♂	Alb. +++; few casts; Exer. 3%	154/80	Moderate	Died	1.0223	12.5	0.652	7.2	0.83
35	25	♀	Alb. ++++; few casts	168/56	Moderate	Died	1.0201	12.5	.....	7.6	0.87
86	40	♂	Alb. ft. tr.; no casts; Exer. 45%	150/80	Slight	Improved	1.0221	12.5	0.560		
90	..	♂	.....	.....	.....	Improved	1.0224	12.5			
96	58	♀	Alb. +++; many casts	145/108	Moderate	Died	1.0236	12.5	0.640	7.3	
102	60	♂	Alb. +; few casts	195/120	None	Improved	1.0246	13.5	0.630	8.1	
23	67	♂	Alb. ++; few casts	195/90	Marked	Died	1.0243	13.5	0.939	7.7	0.75
74	63	♂	Alb. +++; few casts	240/130	Marked	Improved	1.0243	13.5	0.619	7.6	0.74
97	51	♂	Alb. +; no casts	170/100	Moderate	Unimproved	1.0246	13.5	0.650	6.8	
92	..	♂	.....	.....	.....	Improved	1.0215	13.5	0.930	7.9	
46	38	♂	Alb. +; many casts; Exer. 55%	140/90	Slight	Improved	1.0248	13.7	0.620	7.8	0.68
64	56	♂	Alb. ++; occ. cast; Exer. 10%	140/90	None	Died	.....	14.4	0.654	8.3	0.74
50	34	♀	Alb. trace; many casts	208/156	Moderate	Improved	1.0251	15.3	0.630	8.7	0.80
49	39	♀	Alb. ++++; occ. cast; Exer. 50%	185/90	None	Improved	1.0270	15.5	0.602	8.9	0.80
87	40	♂	Alb. +; no casts	176/98	None	Improved	1.0273	16.5	0.580	8.9	
84	34	♀	Alb. +++; many casts; Exer. 20%	190/140	Marked	Dead	1.0290	16.7	0.835	6.8	
81	46	♂	Exer. 8%	230/130	Marked	Dead	1.0290	17.5	0.688	9.9	0.73

Normal Values.—The refractive indexes range from 15.7 to 18.4; specific gravity 1.0258 to 1.030; freezing point lowering 0.550 degrees to 0.620 degrees; solids 8.4 per cent. to 9.9 per cent.; ash 0.75 per cent. to 0.80 per cent.

Chronic Nephritis with Edema.—The refraction, the specific gravity and the solids are very low. A few cases, however, show values well within the normal range. The freezing point lowering is greater than normal, and the ash is normal, pointing toward a dilution of the blood with fluid high in organic crystalloids and normal in inorganic salts.

TABLE 3.—CHRONIC NEPHRITIS WITH UREMIA

Case No.	Age	Sex	Urine	Blood Pressure	Nitrogen Retention	Condition	Specific Gravity	Refractive Index	Freezing Point	Solids, per Cent.	Ash, per Cent.
88	39	♂	Alb. ++++; many casts; Exer. 23%	240/145	Moderate	Improved	1.0234	13.0	0.590		
103	60	♂	Anuria	130/0	Marked	Died	1.0236	12.5	0.830	7.8	
108	26	♂	Alb. +++; occ. cast	260/145	Marked	Slight improvement	1.0243	14.0	0.685	8.2	0.78
47	65	♂	Alb. +++; few casts	.....	.....	Died	1.0254	14.7	0.700	8.3	0.73
85	53	♂	Alb. trace; few casts	230/120	Moderate	Died	1.0247	14.7	0.515	9.1	
109	27	♂	Alb. ++++; no casts; R.B.C. +++	240/140	Marked	Died	1.0273	15.5	0.665	8.7	0.65
48	45	♀	Alb. +++; no casts; Exer. 1%	240/130	Marked	Died	1.0276	15.6	0.676	9.1	0.70
93	..	♀	.....	.....	Marked	Died	1.0264	17.5	0.685	9.1	

The specific gravity and the refraction vary inversely with the degree of edema. Beginning with Case 27 and going to Case 81, we pass from very marked edema to very slight edema, with isolated exceptions. Beginning with Case 102, we find added heart complications. These complications tend to raise the specific gravity and the refraction slightly, because cardiac patients with edema have somewhat higher values than nephritics with edema. From Case 50 and upward, in which the values are normal, we find hypertension in all the cases. This hypertension raises the values from subnormal to normal. In some of the lower cases, hypertension was present, but the edema was much more marked and its effect overshadowed that of the hypertension.

Chronic Nephritis with Uremia.—The refraction is lower than normal, but higher than in nephritis with edema. This is because the blood contains retention products which in themselves have refractive power, and, therefore, increase to a slight extent the refraction of the

otherwise diluted serum. The specific gravity and the total solids are somewhat lower than normal, but much higher than in edematous nephritics, because in uremia the blood is diluted with fluid of urinary composition, while in edema it is diluted primarily with isotonic salt solution. The lowering of the freezing point is usually greater than normal. This is also explained by urinary admixture; the urine having a greater lowering of the freezing point than the serum has. The ash is a little lower than normal, thus suggesting a retention of the organic crystalloids to a much greater extent than the inorganic salts, and, therefore, increasing the absolute but decreasing the relative values.

TABLE 4.—CHRONIC NEPHRITIS WITH EDEMA AND UREMIA

Case No.	Age	Sex	Urine	Blood Pressure	Nitrogen Retention	Condition	Specific Gravity	Refractive Index	Freezing Point	Solids, per Cent.	Ash, per Cent.
22	50	♂	Alb. ++++; many casts; Exer. 20%	240/110	Marked	Died	1.0214	11.4	0.983	6.72	0.74
60	60	♂	Few casts; Exer. 45%	190/100	None	Slight improvement	1.0224	12.9	0.821	7.1	0.70
32	67	♂	Alb. ++; few casts	195/90	Marked	Died	1.0261	14.5	0.713	8.4	0.77
4	23	♂	Alb. ++++; many casts	240/120	Marked	Died	1.0242	14.5	0.720		
3	23	♂	Alb. ++++; many casts	240/120	Marked	Died	1.0256	15.5	0.675		
100	62	♂	Alb. ++++; many casts; many R.B.C.	180/95	Marked	Died	1.0280	16.0	0.790	7.33	
5	23	♂	Alb. ++++; many casts	240/120	Marked	Died	1.0264	16.5	0.770		
13	50	♂	Alb. ++; many casts; Exer. 0%	210/160	Moderate	Unimproved	1.0273	15.5	0.687	9.11	0.89

Chronic Nephritis with Uremia and Edema.—In these cases the refraction is also very low, and the tendency is for a smaller percentage of cases to reach the low normal figures. This is due to greater retention than in either of the foregoing sets of cases, edema alone or uremia alone. If we were to determine the refraction due to the uremic substances, and deduct it from the refraction as usually reported, the resulting figures would fall very much below that found in edema alone. The specific gravity and the solids are low and each seems to be an average of the values found in edema and of that found in uremia. There are many cases, however, whose values run well into the normal figures. The freezing point depression, on the contrary, is always great, due to the fact that large amounts of salts are retained in the blood, and much water also is added due to the

edema. This water ionizes the salts to a much greater extent than in uremia alone, and hence the increased freezing point. This is a good point of differentiation between uremia alone, edema alone, and that of uremia plus edema. The former two have low refractive indexes and low specific gravity, with slightly raised freezing points; the latter have low refractive indexes and low specific gravity, with a marked rise in freezing point. Nothing worthy of note is shown by the ash determination.

In Cases 22 and 60, the edema was marked; in 22 uremia was marked; in 60 the uremia was much less. Case 32 had very slight edema. Numbers 3, 4 and 5 are the same case at different stages. The uremia increased until death. Case 13 is a picture of both marked uremia and edema.

TABLE 5.—Chronic Nephritis without Edema or Uremia

Case No.	Age	Sex	Urine	Blood Pressure	Nitrogen Retention	Condition	Specific Gravity	Refractive Index	Freezing Point	Solids, per Cent.	Ash, per Cent.
31	53	♂	Alb. +++; occ. cast	172/112	.....	Improved	1.0241	13.5	0.709	7.9	0.73
91	30	♂	Alb. 30%; many casts; Excr. trace	180/140	Marked	Died	1.0254	13.8	0.645	10.2	
83	31	♂	Alb. +++; occ. cast	195/145	Moderate	Improved	1.0250	15.0	0.705	8.3	
73	45	♂	Alb. ++++; many casts	210/120	Marked	Unimproved	1.0273	15.6	0.641	9.2	0.80
67	45	♂	Alb. ++++; many casts	280/160	Marked	Unimproved	1.0290	16.5	0.706	9.3	0.76

Chronic Nephritis without Uremia and Edema.—The refraction is somewhat lower than the normal, but higher than that in edema, or that in uremia plus edema. This fact is a great aid in the diagnosis of the foregoing condition. The specific gravity is much the same as in uremia, that is to say, slightly lower than normal, but much higher than the beforementioned nephritic groups. The freezing point has values higher than normal; the solids and the ash are about the same as in normals. In this set of cases it is interesting to note that the specific gravity and the refraction are in no way related to the change in blood pressure.

Cardiac Decompensation with Edema.—In this set of cases all the values are similar to those in nephritis with edema, with the exception that the refraction, the specific gravity and the solids run just a trifle lower in nephritis. The lower these values are, the more marked the edema. With three exceptions, Cases 89 to 16 have nephritis as a secondary diagnosis. Might it not be possible that their edema is due

TABLE 6.—CARDIAC DECOMPENSATION WITH EDEMA

Case No.	Age	Sex	Urine	Blood Pressure	Nitrogen Retention	Condition	Specific Gravity	Refractive Index	Freezing Point	Solids, per Cent.	Ash, per Cent.
89	..	♂	.....	.....	.....	Improved	1.0190	13.0	0.560	6.8	
56	67	♂	Alb. trace; occ. cast; Exer. 10%	200/80	.....	Improved	1.0243	12.5	0.623	7.0	0.78
63	20	♂	Alb. +++; many casts	.....	.....	Died	1.0236	12.5	0.659	7.8	0.71
65	34	♂	Alb. ++++; many casts; Exer. 25%	175/100	Marked	Slight improvement	1.0226	12.5	0.727	7.1	0.75
78	57	♂	Alb. ++; many casts	.....	.....	Improved	1.0225	12.5	0.603	6.9	0.71
98	61	♂	Alb. ft. tr.; no casts	150/100	Slight	Improved	1.0100	12.7	0.596	7.4	
37	26	♀	Alb. ++; no casts	160/70	.....	Died	1.0242	12.8	0.678	7.3	0.81
53	51	♂	Alb. ++; many casts	158/112	Moderate	Improved	1.0235	12.8	0.615	7.4	0.78
38	65	♂	Alb. v. f. tr. no casts; Exer. 35%	180/94	None	Improved	1.0223	13.0	0.672	7.2	0.74
79	65	♂	Alb. trace; occ. cast; Exer. 40%	138/78	.....	Improved	1.0242	13.0	0.597	7.4	0.73
52	42	♀	Alb. +++; few casts	144/110	None	Died	1.0237	13.1	0.633	7.6	0.73
18	60	♂	Alb. trace; few casts	178/110	Slight	Died	1.0235	13.4	0.724	7.2	0.74
16	53	♂	Alb. +++; few casts; Exer. 37%	186/110	Slight	Improved	1.0239	13.5	0.679	7.7	0.67
29	23	♀	Alb. ++; few casts	215/160	Marked	Died	1.0252	13.5	0.816	8.0	0.77
39	41	♂	Alb. +++; no casts; Exer. 31%	170/117	.....	Improved	1.0247	13.5	0.615	7.9	0.76
57	52	♂	Alb. trace; occ. cast; Exer. 13%	120/85	None	Improved	1.0248	13.5	0.920	7.7	0.74
68	49	♂	Alb. ++; occ. cast; Exer. 18%	152/78	None	Improved	1.0254	13.5	0.698	7.7	0.72
30	48	♂	Alb. trace; no casts; Exer. 26%	164/90	None	Improved	1.0250	14.3	0.691	8.0	0.71
44	34	♂	Alb. ++++; many casts; Exer. 25%	175/100	Marked	Slight improvement	1.0250	14.4	0.810	8.2	0.80
45	33	♂	Alb. +++; few casts; Exer. 70%	120/70	Moderate	Died	.....	14.5	0.635	8.6	0.72
24	63	♂	Alb. +; no casts; Exer. 45%	165/120	.....	Died	1.0258	15.4	.....	8.4	0.76

TABLE 6.—Cardiac Decompensation with Edema—(*Continued*)

Case No.	Age	Sex	Urine	Blood Pressure	Nitrogen Retention	Condition	Specific Gravity	Refractive Index	Freezing Point	Solids, per Cent.	Ash, per Cent.
17	64	♂	Alb. +; no casts; Exer. 0%	138/100	Slight	Improved	1.0271	15.5	0.686	8.7	0.66
33	37	♂	.....	220/140	.....	Improved	1.0262	16.5	0.765	6.7	0.74
19	51	♂	Alb. ++++; many casts	135/100	Slight	Dead	1.0289	17.0	0.709	8.34	0.68
21	37	♂	Alb. ++; few casts	190/100	Slight	Dead	.....	17.5	0.825	9.63	0.68
80	58	♂	Alb. trace; occ. cast; Exer. 25%	100/78	Slight	Dead	1.0312	19.4	0.623		
42	42	♂	.....	120/80	Slight	Improved	1.0255	14.7	0.655	8.6	0.76
28	45	♀	.....	.....	.....	Improved	1.0263	15.0	0.555	8.6	0.77

to that condition primarily? If these cases are put into the nephritic group, and those in the nephritic group with cardiac changes as their secondary lesion are put into the cardiac group, a distinct enough line is obtained, which separates the two groups. This grouping could be used in diagnosing cardiopathy and nephropathy. But taking the diagnosis of the clinicians as a basis, the two groups are intermixed.

TABLE 7.—Arteriosclerosis

Case No.	Age	Sex	Urine	Blood Pressure	Nitrogen Retention	Condition	Specific Gravity	Refractive Index	Freezing Point	Solids, per Cent.	Ash, per Cent.
101	60	♂	Alb. ft.tr.; no casts	200/110	.....	Improved	1.0238	13.4	0.620	8.1	
62	35	♂	.....	228/136	.....	Improved	1.0250	14.4	0.770	7.9	0.75
75	52	♂	Alb. ++; many casts; Exer. 0%	120/80	Moderate	Improved	1.0253	14.4	0.715	7.9	0.75
36	56	♂	Alb. ++; no casts	220/90	Slight	Improved	1.0292	14.8	0.807	8.4	0.76
61	50	♂	.....	210/100	None	Improved	1.0270	15.4	0.640	8.5	0.75
12	47	♂	.....	130/60	None	Improved	1.0327	16.0	0.603	9.1	0.82
54	40	♂	Alb. ++; few casts	180/120	None	Improved	1.0282	16.5	0.617	9.4	0.77
99	80	♂	Alb. trace; few casts	230/110	Slight	Improved	1.0280	17.0	0.740	9.3	
55	50	♂	Alb. ++++; few casts	250/150	Slight	Improved	1.0283	17.3	0.879	9.4	0.76
59	58	♂	.....	180/82	.....	Dead	1.0331	19.5	0.633	10.9	0.73

Arteriosclerosis.—In this group we have placed all cases diagnosed primarily as arteriosclerosis, regardless of the blood pressure.

The solids, the refraction and the specific gravity are not always higher than normal, as Butterfield and his co-workers found in their cases. Although some of these cases have much higher values than normals, there are just as many with a subnormal value. The freezing point and ash are the same as in normals.

Case 101 had as a secondary condition, nephritis and edema. Case 36 had cardiac decompensation as a secondary lesion. In these arteriosclerotic cases the values found in no way varied with the blood pressure.

TABLE 8.—ESSENTIAL HYPERTENSION

Case No.	Age	Sex	Urine	Blood Pressure	Nitrogen Retention	Condition	Specific Gravity	Refractive Index	Freezing Point	Solids, per Cent.	Ash, per Cent.
51	63	♂	Alb. v. f. tr.; no casts	235/130	Moderate	Improved	1.0281	16.5	0.725	9.3	0.80
20	44	♀	Alb. ++; few casts; Exer. 50%	270/170	None	Slight improvement	1.0288	17.5	0.670	9.7	0.70
82	47	♂	.....	205/0	None	Improved	1.0292	18.0	0.691	9.7	0.74

Essential Hypertension.—The solids and especially the freezing point, are much higher than normal. The other values are unchanged. This class is very small, only three cases, because we included only those cases whose clinical picture was solely that of hypertension.

Summary, in Chart Form, of Work Done.—In order to bring out to the best advantage the findings in the tables, we have plotted all the results. As ordinates we have the percentage of cases, as abscissae the values of the constant named at the head of each chart. Thus by Chart 7, the lowest curve, that for normals, is meant that 4 per cent. of the total of 23 cases has a refraction of 15.5 or below, 34 per cent. has a refraction of 15.6 to 16.5, 38 per cent. has 16.6 to 17.5, and 22 per cent. has 17.6 to 18.5. The majority of normals, therefore (72 per cent.) have a refraction between 15.6 and 17.5. This form of representation shows vividly how the constants are shifted in the various conditions, and also how much they overlap. Thus, in chronic nephritis with edema, the majority of cases are shifted much to the left (lower values), but some overlap the normal. Wherever the curve does not pass through the points marked by crosses, it is because we always connected the last point with the highest point, for it may be possible that in another set of cases these points may be somewhat higher.

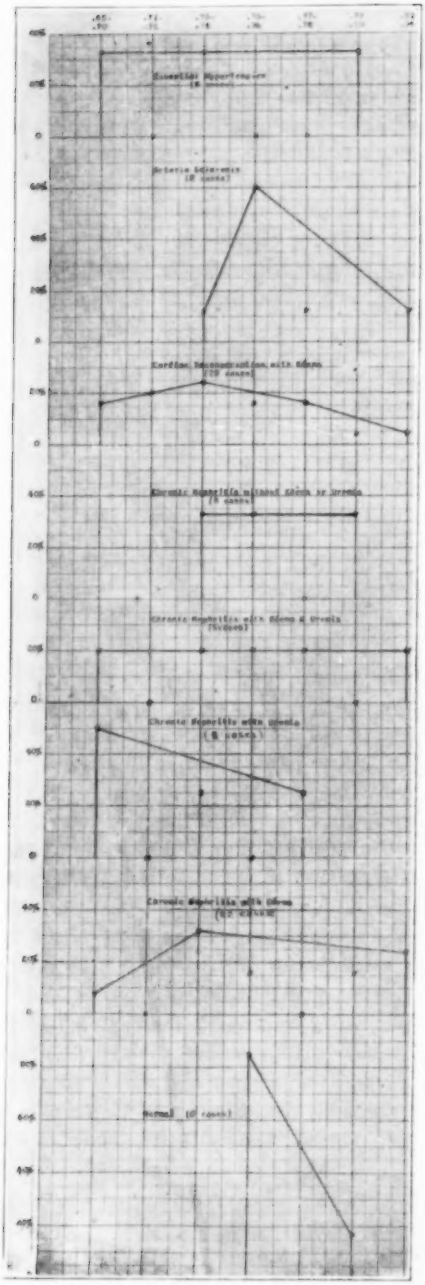

Chart 1.—Refractive index.

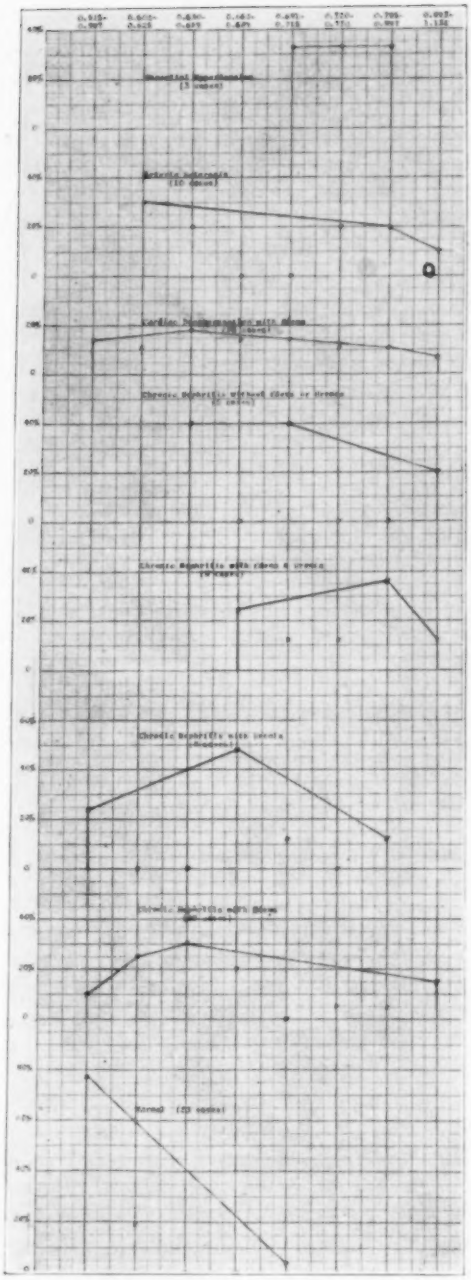

Chart 2.—Solids.

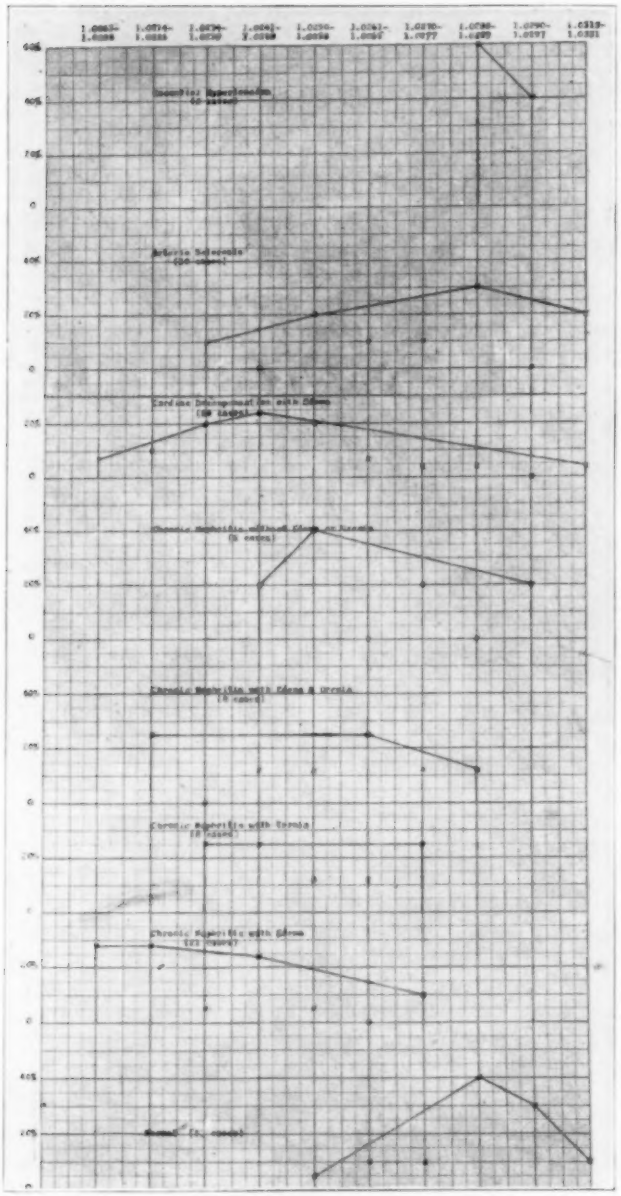

Chart 3.—Specific gravity.

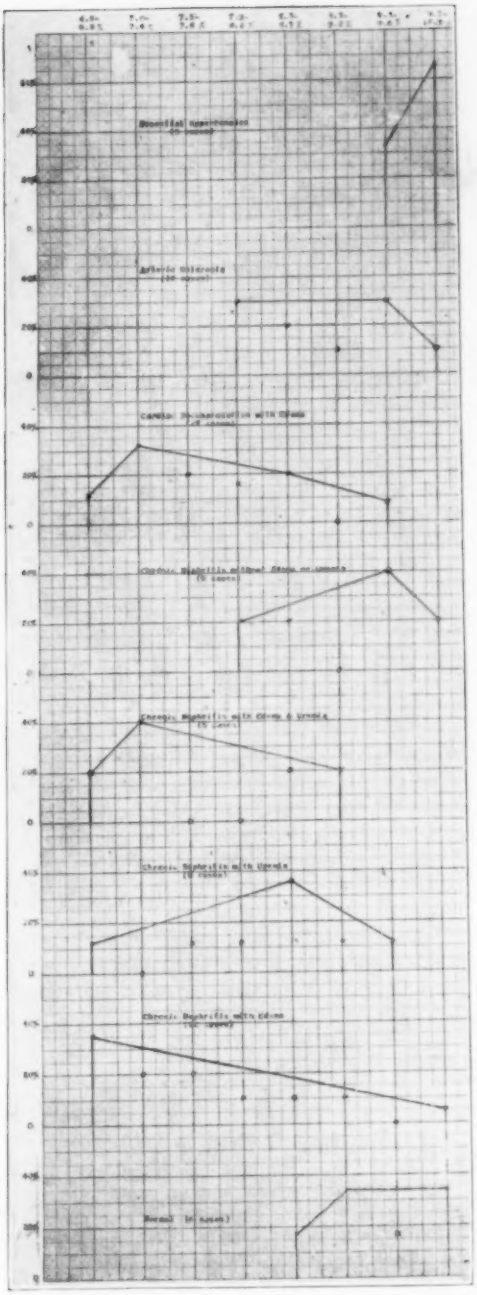

Chart 4.—Freezing point.

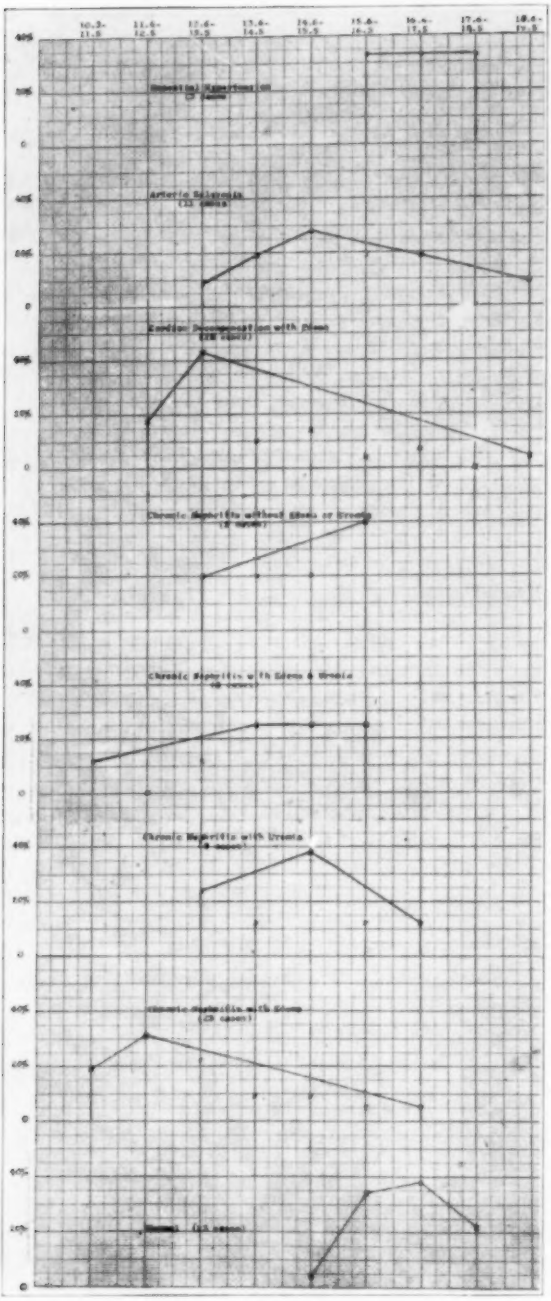

Chart 5.—Ash.

DISCUSSION OF CHARTS

Refraction.—All run lower than normals, except hypertension cases, which are the same as normals. Nephritis with edema is lowest; nephritis with uremia and edema run slightly lower than with uremia alone; nephritis without either uremic or edematous symptoms is a little lower than normal, but higher than the former two; arteriosclerosis is somewhat lower than normal (Chart 1).

Solids.—These run parallel with refraction. This is because the albumin and globulin account for the major portion of the solids (about 80 per cent.), except in nephritis with uremia and edema; here the refraction is more than that which corresponds to the solids. This is due perhaps to the increased uremic substances which affect increasingly the refraction.

A point of differentiation: Nephritis with edema has low solids and low refraction; nephritis with edema and uremia has low solids and higher refraction (Chart 2).

Specific Gravity.—This runs parallel with the preceding two (Chart 3).

Freezing Point.—All types of nephritics have a larger value than normal. Those with edema have values nearest the normal. The blood does not become as concentrated with crystalloids as the other types of nephritis. The cardiac and arteriosclerotic patients have a great range of values, and, therefore, they are of less significance. It is interesting to note that hypertension values are all normal except the freezing point, which is much lowered (Chart 4).

Ash.—Cardiac patients with edema have a lowered inorganic content; nephritic patients without edema, and cases of hypertension, are normal in this respect (Chart 5). Some nephritics with edema have lower ash content and some higher than normal. It is interesting to note that in nephritis with uremia the ash is lower than normal. This suggests that the organic crystalloids and not the inorganic increase in the blood.

SUMMARY

1. Serum values in normal individuals are given for refractive index, specific gravity, freezing point, solids and ash.

2. Determination of these constants, as well as the blood pressure, nitrogen retention and urine picture are reported in chart form in the following classes of cases: Chronic nephritis with edema, with uremia, with edema and uremia, without edema and uremia; cardiacs; arteriosclerosis, and cases of hypertension.

3. The values found in these various conditions overlap each other, and also overlap the normals to a large extent. They show no sharp division as do the cases cited by Butterfield, Erdwurm and Braddock.[1]

4. Charts are inserted which may be of great aid in elucidating questionable cases before a practitioner.

5. Interesting points brought out are the following:

a. Edematous fluid not only collects in tissues, but also dilutes the blood in amount proportional to the edema.

b. Uremic blood is diluted with a fluid of urinary composition. The organic crystalloids seem to be retained to a greater extent than the inorganic salts.

c. In uremia plus edema, more fluid is retained in the blood than in either of the above two classes. This fluid differs from the edematous in being more concentrated with organic substances.

6. Serum pictures:

NEPHRITIS WITH EDEMA	
Refraction	Very low
Specific gravity	Very low
Solids	Very low
Freezing point	Slightly high
Ash	Normal
NEPHRITIS WITH UREMIA	
Refraction	Low
Specific gravity	Slightly low
Solids	Slightly low
Freezing point	Slightly high
Ash	Slightly low
NEPHRITIS WITH UREMIA AND EDEMA	
Refraction	Low
Specific gravity	Low
Solids	Low
Freezing point	Very high
Ash	Not significant
NEPHRITIS WITHOUT UREMIA AND EDEMA	
Refraction	Slightly low
Specific gravity	Slightly low
Solids	Normal
Freezing point	Slightly high
Ash	Normal
CARDIACS WITH EDEMA	
Refraction	Very low
Specific gravity	Very low
Solids	Very low
Freezing point	Normal
Ash	Normal
ARTERIOSCLEROSIS	
Refraction	Not significant
Specific gravity	Not significant
Solids	Not significant
Freezing point	Normal
Ash	Normal
HYPERTENSION	
Refraction	Normal
Specific gravity	Normal
Solids	High
Freezing point	High
Ash	Normal

OBSERVATIONS CONCERNING THE PATHOLOGY OF PANCREATIC FERMENTS*

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INTRODUCTION

At present it is generally recognized that almost all enzymes seem to be toxic when introduced into animal bodies. The first thorough study of the toxicity of enzymes was made by Hildebrandt[1] in 1890. He found that pepsin, diastase, chymosin, emulsin, invertase and myrosin were all toxic; so much so that they produced trembling, uneasiness, difficulty in walking, dyspnea and rising temperature as symptoms. In addition there were numerous hemorrhages from serous and mucous membranes, the lungs, muscles, kidneys, intestines and brain; thrombosis in the body; interference with blood coagulation; fatty degeneration of the myocardium; fatty and parenchymatous degeneration of the liver and hyperemia and parenchymatous and fatty degeneration of the kidney as anatomic changes.

In 1899 Piquet[2] also observed that trypsin and pepsin increased the coagulability of the blood, but after such increase thrombosis frequently occurred.

Morgenroth[3] found that injection of lab ferment subcutaneously caused an abscess at the site of injection. Achalme,[4] in 1901, also observed that the sterilized pancreatic juice injected subcutaneously into guinea-pigs produced a local gelatinous edema followed by gangrene. In 1903, Lombroso[5] found that an inactivated pancreatic juice was much less toxic than the activated.

Noguchi,[6] in 1907, observed that pancreatic lipase is hemolytic when introduced into the blood. In 1911, Kirchheim[7] found that the intravenous injections of pancreatic juice cause death, with lesions in the heart muscle and severe hemorrhage.

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1. Hildebrandt: Virchows Arch. f. path. Anat. **121**: 1890.
2. Piquet: Arch. d. méd. exper., 1899, No. 11.
3. Morgenroth: Centralbl. f. Bakteriol. **26**: 1899, Nos. 11 and 12.
4. Achalme: Ann. de l'Inst. Pasteur **15**: 1901, No. 10.
5. Lombroso: Abstr. in Biochem. Centralbl. **1**: 1903.
6. Noguchi: Biochem. Ztschr. **6**: 1907.
7. Kirchheim: Arch. f. exper. Path. u. Pharmakol. **66**: 1911.

Jobling, Petersen and Eggstein,[8] in 1915, stated that the intravenous injection of trypsin in dogs results in shock similar in many respects to anaphylactic and peptone shock.

My own experiments on the pathology of pancreatin, especially on the toxicity of pancreatin introduced into the circulating blood stream of rabbits, in their results resemble in essential points as to physical symptoms Hildebrandt's observation on the toxicity of various enzymes.

A. TOXICITY OF PANCREATIN

Experiment 1.—For this experiment I employed fifty-three rabbits. Forty-four of these rabbits were injected with a 1 per cent. native pancreatin salt solution filtered through a Berkefeld filter, and the other eight rabbits were injected with a 2 per cent. alcoholic pancreatin salt solution. I injected intravenously 5 c.c. or less of 1 per cent. native pancreatin to 1 kg. of body weight of each rabbit, because any amount over 5 c.c. seemed to be lethal when given intravenously; however, rabbits could resist a 2 per cent. alcoholic pancreatin, about twice the dose of native pancreatin. The injected rabbits were killed at intervals for histologic examination; that is, 4 rabbits were killed at thirty minutes after the injection of the native pancreatin, 3 rabbits at one hour, 4 at one and one-half hours, 3 at two hours, 1 each at two and one-half, six, twelve, and twenty-four hours, 2 at thirty-eight hours, 3 at forty-eight hours, 2 at fifty-two hours, and 1 at each sixty and seventy-two hours; and also 1 each at thirty minutes, one hour, one and one-half hours, three hours, twenty-four hours, and forty-eight hours after the injection of the alcoholic pancreatin.

Procedure for making the pancreatic ferments solutions:

1. Unmodified pancreatic ferments solutions.—Commercial pancreatin (P. D. & Co), trypsin (Central Scientific Supply Company) and amylopsin (Digestive Ferments Company) powders were added to physiologic sodium chlorid solution in the amount of 1 per cent. by weight. After repeated shaking the solutions were filtered through hard-pressed filters and finally through Berkefeld candles. The resulting filtrate was clear and sterile.

2. Alcohol modified pancreatic ferment solutions.—Ten per cent. solutions of the ferments mentioned were made in physiologic sodium chlorid solution, and after thorough shaking were filtered through hard-pressed paper. To the resulting filtrate were added 20 volumes of absolute alcohol and the mixture allowed to stand thirty minutes, with the formation of a white precipitate resulting. The mixture was then centrifugalized and the sediment rapidly resuspended in an amount of salt solution making the concentration 2 per cent. relative to the amount of pancreatic ferments originally employed.

All of the rabbits experimented on showed such physical symptoms as trembling, uneasiness, difficulty in walking, dyspnea and strong palpitation of the heart, and sometimes shock a short time after the injection; however, in the case of the alcohol modified pancreatin the degree of these symptoms was much less than in the animals given native pancreatin, corresponding to Lombroso's observation on pancreatic juice.

B. PATHOLOGIC CHANGES

1. The Production of Hyperemia, Hemorrhage and Thrombosis.—Thirty-three rabbits were employed, twenty-seven of which were intravenously injected once with native pancreatin. The other six rabbits were injected with an alcoholic solution of pancreatin. These rabbits were killed for anatomic observation.

8. Jobling, Petersen and Eggstein: J. Exper. M. **22**: 1915, No. 2.

The rabbits developed more or less hyperemia, hemorrhage and thrombosis throughout the body in almost all instances, but the production of thrombosis was mainly found in liver and lung, although kidney and bone marrow showed it in a few cases. Hemorrhagic inflammation seemed to be markedly produced after one hour following the injection of antigen. The hemorrhagic foci showed a condition of absorption at thirty-eight hours after the injection. This hemorrhagic phenomenon was produced not only in liver, spleen, lung, kidney, bone marrow, heart muscle, pancreas and suprarenal capsule, but also in the abdominal cavity in four cases, in the omentum in five cases, in the urine in one case, in the abdominal muscles in three cases, and in the superficial muscles of the thigh in two cases.

In the alcoholic pancreatin cases the anatomic changes were almost the same as in the cases of native pancreatin injection, even though the physical symptoms, compared with the former, indicated less toxicity.

Of these inflammations, there were found in many cases an infiltration of small round cells in Glisson's capsule and atrophy of the liver cells, which latter was due, no doubt, to the pressure of the hemorrhage. This was noticeable in one case in particular in which the animal was killed fifty-four hours after the injection. In the kidneys the hemorrhagic lesions were found chiefly in the glomeruli and in Henle's loop. Hyaline casts were also found in many cases. The kidneys presented an acute hemorrhagic nephritis, so that the urines indicated a markedly positive protein reaction of the rabbits which were killed before forty-eight hours after the injection. The protein reaction, however, was not found in the rabbits killed after fifty-four hours following the injection. In the pancreas the hemorrhagic lesions were marked in the islands of Langerhans, although they were found in other parts.

On the coagulation time of the blood after the injection of enzymes, as in Hildebrandt's and Piquet's observations on various enzymes, I also found delay in two cases of twenty-seven injected intravenously with native pancreatin. In animals killed at two and forty-eight hours after the injection coagulation of the blood was very slow.

C. PATHOLOGIC CHANGES IN RABBITS IMMUNIZED WITH PANCREATIN

In 1899, Schepilewsky[9] produced extensive subcutaneous necrosis and suppuration by the injection of various kinds of ferments, such as lab ferment, pancreatin, papayotin, etc., and succeeded in causing an amyloid degeneration in rabbits; that is, two (in spleen, intestine

9. Schepilewsky: Centralbl. f. Bakteriol. **25**: 1899.

and liver) of seven cases with lab ferment, one (in spleen) of six cases with pancreatin, and one (in spleen) of two cases with papayotin; he concluded that the injection of these enzymes caused hyaline degeneration more frequently than amyloid degeneration. In 1903, Wells[10] observed fat necrosis in the omentum and mesentery of dogs and cats after the intraperitoneal injection of pancreatin. He concluded that the lipase in pancreatin may be the agent causing the fat necrosis. This phenomenon can be produced with constancy in dogs and cats, but less successfully in rabbits.

I also observed various pathologic changes in experimental rabbits immunized with pancreatin.

For this experiment I employed twelve rabbits immunized with native or an alcoholic pancreatin.

As already shown in the foregoing experiments toxicity was produced in the rabbits by the injection of the antigen up to five or six injections; however, the toxicity decreased after the promoting of the immune process in the rabbits. This phenomenon was found more marked after intravenous than after subcutaneous or intraperitoneal injections. By subcutaneous injection there was produced an abscess at the site of the injection, but no fat necrosis intraperitoneally. In intravenous injections, if a small amount of the antigen reaches the extravascular tissue it produces locally an acute inflammation followed by gangrene. In addition, some of these rabbits developed during the immunization a crippled condition in the fore legs or hind legs, especially in the hind legs, together with a loss of body weight.

In the histologic examination I found many pathologic changes in the organs, as shown in the following protocol.

PROTOCOL

EXPERIMENT 2.—*Macroscopic anatomic changes in rabbits immunized with native pancreatin.*—In this experiment three rabbits were employed.

Rabbit 11 (Male).—Total amount of antigen injected, 100 c.c. of 1 per cent. pancreatin (injected seven times intravenously and three times peritoneally)—1 gm.; animal killed on the sixty-first day after the first injection (eighth day after the last injection); there was found no loss of body weight.

Necropsy.—All organs seemed in normal condition.

Rabbit 24 (Female).—Total amount of antigen injected, 72 c.c. of 1 per cent. pancreatin (injected nine times into the ear vein)—0.72 gm.; killed on the fifty-first day after the first injection (tenth day after the last injection); there was sometimes produced an acute inflammation followed by necrosis, which was due to the small amount of antigen that went into the extravascular tissues of the external part of the ear during the immunization; a crippled condition was found in the left fore leg on the fifty-first day and in the hind legs on the fifty-fourth day after the first injection; body weight was markedly decreased.

Necropsy.—An enlargement of the spleen was found; the bone marrow of the legs showed a yellow, grayish-white color, and a moderate hardening, especially of the hind legs.

10. Wells: J. M. Res. **9**: 1903.

Rabbit 25 (*Female*).—Total amount of antigen injected, 80 c.c. of 1 per cent. pancreatin (injected ten times into the ear vein)—0.8 gm.; killed on the fifty-sixth day after the first injection (tenth day after the last injection); a crippled condition in the hind legs was found on the forty-eighth day after the first injection; in this case also an acute inflammation followed by gangrene was sometimes produced in the extravascular tissue of the external part of the ear where the antigen was injected intravenously; body weight was markedly decreased.

Necropsy.—An enlargement of the spleen and both suprarenal glands was found; also hardness of the bone marrow of both hind legs, which was brownish white in color; there was no change in the bone marrow of the fore legs. Large abscesses were found in the subcutaneous tissue surrounding both hip joints; the lesions were old, showing softness in consistence and a milky white color.

EXPERIMENT 3.—*Macroscopic anatomic changes in rabbits immunized with an alcoholic pancreatin solution.*—Six rabbits were employed in this experiment.

Rabbit 1 (*Male*).—Total amount of the antigen injected, 95 c.c. of a 2 per cent. alcoholic pancreatin (injected ten times intravenously and once peritoneally)—1.9 gm.; died on the fifty-fourth day after the first injection (eighth day after the last injection); a crippled condition was found in both hind legs on the thirty-sixth day after the first injection; body weight was markedly decreased.

Necropsy.—A slight enlargement of the spleen was found; hardness of the bone marrow of both hind legs, and a yellowish-gray color and also a slight hardness and a brownish gray in color of that of the both fore legs.

Rabbit 2 (*Female*).—Total amount of the antigen injected, 110 c.c. of a 2 per cent. alcoholic pancreatin (injected ten times into the ear vein and twice peritoneally)—2.2 gm.; killed on the sixty-third day after the first injection (eighth day after the last injection); there was produced an acute inflammation followed by necrosis on the external part of the ear where some of the antigen injected intravenously entered the extravascular tissue; body weight was slightly decreased.

Necropsy.—A slight enlargement of the spleen and both suprarenal glands; hardness and a grayish-white color of the bone marrow of the legs.

Rabbit 4 (*Male*).—Total amount of the antigen injected, 108 c.c. of a 2 per cent. alcoholic pancreatin (injected eleven times intravenously and once peritoneally)—2.16 gm.; killed on the sixty-first day after the first injection (eighth day after the last injection); body weight slightly decreased.

Necropsy.—An enlargement of the spleen; hardness and a grayish-white color of the bone marrow of both hind legs but no change of that of the fore legs.

Rabbit 5 (*Male*).—Total amount of the antigen injected, 77 c.c. (injected ten times intravenously and twice peritoneally)—1.54 gm.; killed on the sixty-first day after the first injection (eighth day after the last injection); a crippled condition was found in the left hind leg on the forty-ninth day after the first injection; body weight was markedly decreased.

Necropsy.—Body markedly emaciated; a hemorrhagic infarct of the lower lobe of the left lung, and a hemorrhage of the lower lobe of the right lung; jaundice; that is, liver, fat tissue of the mesentery, conjunctivae and urine were yellow in color; hardness and a yellow-grayish white color of the bone marrow of the left hind leg; the urine showed positive for bile pigment by Gmelin's method.

Rabbit 6 (*Female*).—Total amount of antigen injected, 104 c.c. of a 2 per cent. alcoholic pancreatin (injected three times intravenously, three times subcutaneously and five times peritoneally)—2.08 gm.; killed on the sixty-fifth day after the first injection (tenth day after the last injection); a tumor was found on the sixteenth day after the injection, in the subcutaneous tissue of

the abdominal wall where the antigen was injected subcutaneously; this tumor was the size of a nut and of a moderate hardness; body weight was not changed.

Necropsy.—Enlargement of the spleen and both suprarenals; hardness of the bone marrow of the thighs of both hind legs, but no change of that of the lower legs. The tumor in the abdominal wall was of a yellowish, milky-white color and sticky, but had no odor.

Rabbit 8 (Male).—Total amount of antigen injected, 64 c.c. of a 2 per cent. alcoholic pancreatin (injected seven times intravenously and twice peritoneally) —1.28 gm.; killed on the fifty-sixth day after the first injection (ninth day after the last injection); body weight slightly decreased.

Necropsy.—An enlargement of the spleen; no change of the bone marrow.

EXPERIMENT 4.—*Macroscopic anatomic changes in rabbits immunized with a native and an alcoholic pancreatin.*—In this experiment three rabbits were employed.

Rabbit 3 (Female).—Total amount of antigen injected, 15 c.c. of a 1 per cent. native pancreatin (three times intravenously), and 42 c.c. of a 2 per cent. alcoholic pancreatin (eight times intravenously)—0.99 gm. altogether; killed on the sixty-third day after the first injection (eighth day after the last injection); body weight was slightly decreased.

Necropsy.—Body was emaciated; enlargement of the spleen and both suprarenals; liver cirrhosis; no change in the bone marrow.

Rabbit 9 (Female).—Total amount of antigen injected, 27 c.c. of 1 per cent. native pancreatin (three times intravenously) and forty-one c.c. of 2 per cent. alcoholic pancreatin (twice intravenously and three times peritoneally)—1.09 gm.; killed on the sixty-first day after the first injection (eighth day after the last injection); a crippled condition was found in the left hind leg on the twenty-first day after the first injection; body weight was not changed.

Necropsy.—All organs seemed in normal condition.

Rabbit 10 (Female).—Total amount of the antigen injected, 24 c.c. of a 1 per cent. native pancreatin (three times intravenously) and 36 c.c. of a 2 per cent. alcoholic pancreatin (twice intravenously and three times peritoneally)—0.96 gm.; killed on the sixty-first day after the first injection (eighth day after the last injection); body weight was moderately increased.

Necropsy.—An enlargement of the spleen; the other organs seemed in normal condition.

Histologic Examination.—Materials obtained from the necropsies were fixed in Zenker's fluid and formaldehyd solution, and sections were prepared by the paraffin and frozen section methods. These sections were treated with the following staining solutions:

1. Hematoxylin-eosin.
2. Van Gieson's picro-fuchsin and iron-hematoxylin.
3. Gentian-violet.
4. Malachite-green, acid fuchsin, and martius yellow (Pianese's[11] staining solution for the differentiation of hyalin, colloid, mucin and a substance resembling amyloid).
5. Sudan III-hematoxylin (employed for the liver, kidneys, spleen myocardium and lungs).

The following results were obtained microscopically:

Liver: All the livers of twelve rabbits showed a greater or less increase in Periportal and Glisson's capsule connective tissue with an infiltration of small round cells in those areas; in addition, Rabbit 1 showed many hemorrhagic lesions; Rabbits 3 and 5 showed many thrombi in the smaller portal veins and hyaline degeneration of some of these thrombi; Rabbit 8 showed a typical liver cirrhosis.

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11. Pianese: Mallory and Wrights Pathological Technique, Ed. 6, 1915, p. 306.

The media of median portal veins of the liver stained a deep blue with hematoxylin-eosin in Rabbit 25.

Tawara,[12] in 1913, also found such a substance stained a deep blue with hematoxylin-sudan in the intima of some arteriosclerotic vessels, and he decided that the substance was hyalin. The substance fund in the media of the portal veins in my experiment, therefore, corresponds to his substance found in the intima by staining; also the substance in my experiment stained a brick-red color with Pianese's solution, so that this change of the media of the portal veins probably is a hyaline degeneration.

Liver cells surrounding the acini in most cases, and also those surrounding the portal veins in some other cases, presented, in general, a slight swelling; however, the cells of one case (Case 24) were vacuolated and in a degenerated condition; that is, the cells stained an intense red with eosin, but in opacity resembled hyaline degeneration; they also stained a yellowish brown with Van Gieson's picro-fuchsin. The liver cells of five rabbits (3, 4, 8, 24 and 25) were stained a dark reddish violet with gentian-violet. Those of other rabbits (5, 9 and 10) were stained a brick-red in some cells and (slightly) a dark reddish violet in others, but a dark green with gentian-violet. Those of three other rabbits (1, 2 and 6) stained a brick red with Pianese's staining solution, and a dark green with gentian-violet, while the liver cells of Rabbit 11 seemed in a normal condition.

Considering the degeneration of liver cells from the results of staining, the cells of five rabbits (3, 4, 8, 24 and 25) the condition seemed to be neither a hyaline, colloid nor a typical amyloid degeneration, but seemed to be an "amyloid-like" degeneration, while the liver cells of three other rabbits (5, 9 and 10) were certainly in an intermediary condition between hyaline and amyloid degeneration; and also those of three other rabbits (1, 2 and 6) were in a state of hyaline degeneration. The degeneration of these liver cells was most marked in two rabbits (24 and 25), moderate in six rabbits (3, 4, 5, 6, 8, 9 and 10), and slight in three rabbits (1, 2, and 6); especially the liver cells of the Rabbits 24 and 25 were markedly swollen and some nuclei were displaced or had disappeared. Frozen sections of these liver tissues were also stained with sudan III and in all cases showed a positive reaction. The positive reaction of sudan III for fatty degeneration was shown in the instance of a slight degeneration in the liver cells surrounding the acini and in the Kupffer cells; however, it was best exhibited in marked degeneration in areas of acini and in Glisson's capsule. The degree of fatty degeneration was moderate in nine rabbits (1, 2, 3, 4, 5, 9, 11, 24 and 25) and marked in three rabbits (6, 8 and 10).

Kidneys: The kidneys also showed greater or less degenerative changes in the tubular epithelium in all cases, suggesting a granular degeneration, but most marked in the convoluted tubules; some desquamation of tubular and glomerular epithelium; formation of casts; a slight hyperemia; some interstitial infiltration with small round cells; a slight cellular proliferation of connective tissue, and also hemorrhagic lesions, in some cases showing an acute or subacute parenchymatous degeneration and glomerulitis.

Some of the epithelial cells of the convoluted tubules and glomeruli were stained an intense red color with eosin, resembling hyaline degeneration; in some cases they were stained a dark red with Pianese's staining solution, a slight reddish violet with gentian-violet and a yellowish brown with Van Gieson's picro-fuchsin. The cells of six (1, 3, 5 6, 11 and 25) of twelve rabbits, were most marked in the convoluted tubules, being stained a dark red with Pianese's solution and a slight reddish violet with gentian-violet. In one case (6) of these six rabbits hyalin-like substance was found as large masses in the glomeruli; in two other cases (2 and 4) of twelve rabbits two kinds of degeneration were shown in the epithelial cells of the convoluted tubules and glomeruli. Some epithelial cells were stained a brick-red color with

12. Tawara: Verhandl. d. Jap. path. Gesellsch. **3**: 1913.

Pianese's solution but a dark green with gentian-violet, and some were of a dark-red color with Pianese's solution and a slight reddish violet with gentian-violet; in three other cases (8, 9 and 10) they were stained a brick red with Pianese's solution and a dark green with gentian-violet, while in one case (24) there was not found any special staining reaction with Pianese's solution and gentian-violet.

In summary, the degenerated epithelial cells, therefore, showed an amyloid-like degeneration in six rabbits (1, 3, 5, 6, 11 and 25), a hyaline and an amyloid-like degeneration in two rabbits (2 and 4), and a hyaline degeneration in three other rabbits (8, 9 and 10).

Frozen sections stained with sudan III showed, in general, a fatty degeneration in all the cases, affecting principally the convoluted tubules, although the glomeruli were slightly affected. The degree of this degeneration was slight in two cases (3 and 9), moderate in six other cases (1, 2, 4, 5, 11 and 25) and strong in four more cases (6, 8, 10 and 24).

Spleen: The spleens showed, in general, a moderate increase of phagocyte and giant cells in the pulp, and also an increase of lymphoid tissue and the connective tissue framework; hyperemia in all cases, and some hemorrhagic lesions in other cases.

Some special pathologic changes presented a hyalin-like substance in the pulp or malpighian bodies, which stained a dark-red color with Pianese's solution, and a dark green with gentian-violet. The deposit of this substance was recognized in ten cases (1, 2, 3, 4, 5, 6, 9, 10, 24 and 25) out of twelve rabbits; however, it was most remarkable in three (3, 4 and 5) of these cases, especially in Rabbit 3, in which the hyalin-like substance was deposited in the endothelial cells of a capillary in a malpighian body, but was not found in the other cases.

The hyalin-like or amyloid-like degeneration of the cells of the spleen was principally recognized in macrophages and giant cells, and also slightly in endothelial cells of the reticular tissue, while in two cases of Rabbits 8 and 10 no such change was recognized. The frozen sections, stained with sudan III, showed a positive result in all the cases; that is, very slight in one case (25), slight in eight other cases (1, 2, 3, 8, 9, 10, 11 and 24), moderate in two cases (4 and 5) and strong in one case (6).

Heart Muscle: The heart muscle of six rabbits (2, 3, 9, 10, 11 and 24) showed hemorrhagic lesions; some increase of interstitial connective tissue in two rabbits (2 and 8), and some atrophy in five rabbits (2, 8, 9, 10 and 11). Some pathologic changes took an intensely red color with eosin, and a slightly reddish violet with Pianese's solution, but a dark green with gentian-violet, showing moderately an amyloid-like degeneration in five rabbits (3, 4, 8, 9 and 10). There were a hyalin-like degeneration in five other rabbits (2, 5, 6, 11 and 25), while there was no such change in two cases (1 and 24).

Frozen sections stained with sudan III showed a fatty degeneration in all the cases; very slight in eight rabbits (1, 2, 3, 8 9, 11, 24 and 25), moderate in three rabbits (4, 5 and 10) and strong in one rabbit (6).

Lungs: The lungs showed some increase of interstitial connective tissue accompanied by infiltration of small round cells in ten cases (1, 2, 3, 4, 8, 9, 10, 11, 24 and 25); in twelve rabbits a moderate hyperemia in five cases (1, 2, 6, 9 and 11), and some hemorrhagic lesions in two cases (5 and 24). Rabbit 5 showed a marked hemorrhagic infarct, a thickening of the intima and some increase of the media of some of the smaller or medium sized pulmonary arteries in four cases (5, 11, 24 and 25), and some thrombi in the capillaries in three cases (3, 24 and 25). A hyalin-like or an amyloid-like degeneration, as found in other organs, was not at all present with special stains. Frozen sections stained with sudan III showed no fatty degeneration.

Bone Marrow: The necropsies on seven rabbits (1, 2, 4, 5, 6, 24 and 25) showed amyloid degeneration, and five other rabbits (3, 8, 9, 10 and 11) showed a normal condition. Twelve rabbits showed histologically a pathologic con-

dition. Seven rabbits (1, 2, 4, 5, 6, 24 and 25) showed a necrotic condition of marrow cells and also five more rabbits (3, 8, 9, 10 and 11) showed, in general, a degenerative condition of marrow cells but an entirely necrotic condition showing karyolysis and karyorhexis at some parts. Fibrous and reticular tissue was fairly proliferated, especially in three rabbits (1, 5 and 25), together with cicatricial tissue. These marrow cells generally stained an intensely red color with eosin, a reddish violet with a gentian-violet and a slight reddish-violet color with Pianese's solution, especially the giant cells and large marrow cells, and also a yellowish brown with Van Gieson's picro-fuchsin.

Pancreas: The pancreas in all the cases were in almost a normal condition; however, a slight hyperemia was found in three cases (1, 6 and 11), and also a slight increase of the interstitial connective tissue in four cases (3, 8, 9 and 11).

Suprarenal Glands: The adrenals of all the animals were in normal condition.

D. COMPARATIVE STUDIES ON THE PATHOLOGY OF PANCREATIN, TRYPSIN AND AMYLOPSIN

For this experiment I employed seven rabbits immunized with a native or an alcoholic trypsin and amylopsin. The clinical symptoms produced in rabbits were just the same as in the instance of the pancreatin. It is not necessary to go into detail in regard to the toxicity of the trypsin and amylopsin.

PROTOCOLS OF EXPERIMENTS

EXPERIMENT 5.—*Macroscopic anatomic changes of rabbits immunized with a native trypsin.*—In this experiment two rabbits were employed.

Rabbit 20 (Male).—Total amount of antigen injected, 75 c.c. of a 1 per cent. trypsin (injected eight times intravenously and once peritoneally)—0.75 gm.; killed on the forty-second day after the first injection (eighth day after the last injection); body weight was slightly decreased.

Necropsy.—A slight jaundice (a slight yellow color of liver, intestine and urine). The urine was positive for bile pigment by Gmelin's test.

Rabbit 21 (Female).—Total amount of antigen injected, 85 c.c. of a 1 per cent. trypsin (injected eight times intravenously and once peritoneally)—0.85 gm.; killed on the one hundred and nineteenth day after the first injection (eighty-sixth day after the last injection); body weight was slightly decreased up to the last injection; however, afterward it gradually increased.

Necropsy.—An enlargement of the spleen and both kidneys; congestion of the kidneys and bone marrow; a remarkable yellow color of the liver.

EXPERIMENT 6.—*Macroscopic anatomic changes in rabbits immunized with alcoholic trypsin.*—In this experiment one rabbit was used.

Rabbit 22 (Female).—Total amount of the antigen injected, 51 c.c. of a 2 per cent. alcoholic trypsin (injected eight times intravenously and once peritoneally)—1.02 gm.; killed on the one hundred and nineteenth day after the first injection (eighty-sixth day after the last injection); body weight slightly decreased up to the last injection, but later gradually increased.

Necropsy.—A remarkable yellow color of the liver; congestion of the bone marrow.

EXPERIMENT 5.—*Macroscopic anatomic changes in rabbits immunized with native amylopsin.*—In this experiment two rabbits were employed.

Rabbit 16 (Male).—Total amount of antigen injected, 87 c.c. of 1 per cent. amylopsin (injected eight times intravenously and once peritoneally)—0.87 gm.; killed on the one hundred and twenty-first day after the first injection (eighty-

sixth day after the last injection); there was almost no change in the body weight.

Necropsy.—An enlargement of the suprarenal glands; a yellow color of the liver and myocardium; hardness of the bone marrow of both thighs and showing a yellow-gray color.

Rabbit 17 (Female).—Total amount of the antigen injected, 82.5 c.c. of 1 per cent. amylopsin (injected eight times intravenously and once peritoneally); killed on the forty-fourth day after the first injection (ninth day after the last injection); body weight was gradually decreased.

Necropsy.—Congestion of the lower lobes of both lungs, kidneys and spleen; hardness of bone marrow of both thighs, showing a grayish-white color.

EXPERIMENT 8.—*Macroscopic anatomic changes in rabbits immunized with alcoholic amylopsin.*—In this experiment two rabbits were employed.

Rabbit 18 (Male).—Total amount of antigen injected, 67.5 c.c. of 2 per cent. alcoholic amylopsin (injected eight times intravenously and once peritoneally)—1.35 gm.; killed on the forty-fourth day after the first injection (ninth day after the last injection); body weight was slightly increased.

Necropsy.—Congestion of the lower lobes of both lungs; hemorrhagic infarct of the spleen; no change in the bone marrow.

Rabbit 19 (Female).—Total amount of antigen injected, 71.5 c.c. of 2 per cent. alcoholic amylopsin (injected eight times intravenously and once peritoneally)—1.43 gm.; killed on the one hundred and twenty-first day after the first injection (eighty-sixth day after the last injection); body weight was slightly decreased up to the last injection; however, it afterward gradually increased.

Necropsy.—All organs seemed in normal condition.

Histological Examination.—Materials obtained at necropsy from all the animals were treated the same as in the instance of the pancreatin.

A. The findings in the case of the rabbits immunized with trypsin were as follows:

Liver: The livers of three rabbits showed a greater or less increase in periportal and Glisson's capsule connective tissue, with an infiltration of small round cells in those areas, Rabbit 21 especially showing a typical liver cirrhosis, and also Rabbit 20 showing a slight congestion. Liver cells surrounding the acini in all cases showed some degenerative condition in the cases of Rabbits 21 and 22 especially, staining an intensely red color with eosin, a dark reddish violet with Pianese's solution, and a yellowish brown with Van Gieson's picro-fuchsin, while a dark green was produced with gentian-violet, thus showing an amyloid-like degeneration. Frozen sections stained with sudan III showed a fatty degeneration, moderate in two cases (20 and 21) and strong in one case (22).

Kidneys: The kidneys presented a greater or less degenerative change in the tubular epithelium in all cases, showing granular degeneration especially marked in the convoluted tubules; and also some desquamation of tubular and glomerular epithelium, formation of casts, a slight hyperemia and some increase of the interstitial connective tissue, with an infiltration of small round cells.

Some epithelial cells of the convoluted tubules and glomeruli were stained an intensely red color with eosin, a dark-red color with Pianese's solution, and a yellowish brown with Van Gieson's picro-fuchsin, but a dark green with gentian-violet, indicating an amyloid-like degeneration of these cells. This change was markedy present in two cases, Rabbits 21 and 22, while in one case, Rabbit 20, it looked similar to hyaline degeneration.

Frozen sections stained with sudan III showed fatty degeneration, very slight in Rabbit 20, moderate in Rabbit 21, and strong in Rabbit 22.

Spleen: The spleens showed a moderate increase of phagocytes and giant cells in the pulp, the lymphoid tissue and the connective tissue framework, with hyperemia in all cases.

In the special pathologic changes there was presented a hyalin-like or an amyloid-like substance in the pulp or malpighian bodies, which stained an intensely red color with eosin in all cases, a dark-red color or a slight reddish violet with Pianese's solution in two cases (Rabbits 21 and 22), but a brick color in one case (Rabbit 20), a yellowish brown with Van Gieson's picro-fuchsin and a dark green with gentian-violet, thus showing an amyloid-like degeneration in two cases (21 and 22) and a hyalin-like degeneration in one case (20).

Such a change was chiefly recognized in phagocytic and giant cells and also slightly in endothelial cells of reticular tissue.

Frozen sections stained with sudan III showed a slight fatty degeneration in two cases (20 and 21), and moderate in one case (22).

Myocardium: The myocardium in all cases showed some hemorrhagic lesions, some increase of the interstitial connective tissue, some atrophy in one case (22), and some hyalin-like degeneration which stained an intensely red color with eosin, a brick color with Pianese's solution and a dark green with gentian-violet.

Frozen sections stained with sudan III showed fatty degeneration in all cases.

Lungs: The lungs showed some increase of interstitial connective tissue and an infiltration of small round cells, some hyperemia, and thrombosis of the smaller pulmonary veins in all cases, and a marked thickening of the intima and some increase of the media of smaller or medium sized pulmonary arteries in two cases (20 and 21), with no pathologic changes in the cells.

Frozen sections stained with sudan III showed no fatty degeneration.

Bone Marrow: The bone marrow showed a slight hyperemia in all the cases; in two cases (20 and 21) there was, in general, a normal condition of marrow cells, although there was present a slight hyalin-like degeneration of a few large marrow cells and giant cells. In one case (22) there were observed necrotic areas and some granulation tissue in such areas. There were also present many large marrow and giant cells in an amyloid-like degeneration, staining an intensely red color with eosin, a slight reddish violet with Pianese's solution and gentian-violet, and also a yellowish brown with Van Gieson's picro-fuchsin.

Pancreas: The pancreas in all the cases were in almost normal condition although a slight hyperemia was found.

Suprarenal Glands: All adrenals were in a normal condition.

B. The findings in rabbits immunized with the amylopsin were as follows:

Liver: The livers of four rabbits showed a greater or less increase of periportal and Glisson's capsule connective tissue, with an infiltration of small round cells and a slight hyperemia. Rabbit 16, showed some thickening of the intima, together with an increase of the media of the smaller hepatic arteries. Rabbits 17 and 18 showed an increase of bile ducts. The liver cells surrounding the acini in all the cases showed some degeneration, especially remarkable in the cases of Rabbits 16 and 19; staining an intensely red color with eosin, a dark reddish violet with Pianese's solution, a yellowish brown with Van Gieson's picro-fuchsin, and a dark green with gentian-violet, just suggesting an amyloid-like degeneration.

Frozen section stained with sudan III showed considerable fatty degeneration in all the cases.

Kidneys: The kidneys showed a greater or less degenerative change in the tubular epithelium in all the cases, a granular degeneration, especially remarkable in the convoluted tubules, some desquamation of tubular and glomerular epithelium, formation of casts, a slight hyperemia and some increase in the interstitial connective tissue, with an infiltration of small round cells. Rabbit 17 showed some thickening of the intima, together with an increase of the media of the smaller renal arteries.

Some epithelial cells of the convoluted tubules and glomeruli, especially the convoluted tubules of three rabbits (16, 17 and 19) were stained an intensely

red color with eosin, a dark reddish violet with Pianese's solution, a yellowish brown with Van Gieson's picro-fuchsin, and a dark green with gentian-violet, indicating an amyloid-like degeneration of the epithelial cells. Rabbit 18 showed no such change.

Frozen sections stained with sudan III showed a marked fatty degeneration in all the cases.

Spleen: The spleens showed an increase of phagocyte and giant cells in the pulp, the lymphoid tissue and connective tissue framework, and some hyperemia in all the cases. Rabbit 16 showed some thickening of the intima of the smaller splenic arteries. No other special change was present in all the cases. Frozen sections stained with sudan III showed a marked fatty degeneration in one case (16) and slight in three cases (17, 18 and 19).

Myocardium: The heart muscle in all the cases showed some hemorrhagic lesions, and some thrombosis in one case (16); and a hyaline degeneration of the myocardium in one case (17), which stained an intensely red color with eosin and a brick-red color with Pianese's solution. In three other rabbits no such change was present.

Frozen sections stained with sudan III showed much fatty degeneration in all the cases.

Lungs: The lungs showed some increase of the interstitial connective tissue, with an infiltration of small round cells and some hyperemia in all the cases. Marked thickening of the intima and some increase of the media of the smaller or the medium sized pulmonary arteries were found in three cases (16, 17, and 18), and also an amyloid-like degeneration of the media of the medium pulmonary arteries in one case (19), which showed a characteristic staining reaction with eosin and Pianese's solution, as in the other instances.

Frozen sections stained with sudan III showed no fatty degeneration.

Bone Marrow: The bone marrow of two cases (16 and 17) showed some necrosis. The large marrow cells and giant cells of all the cases showed generally an amyloid-like degeneration, especially remarkable in two cases (16 and 17), and a characteristic staining reaction with eosin and Pianese's solution and gentian-violet, as in the other instances.

Pancreas: The pancreas of Rabbit 16 showed a hyalin-like degeneration of the epithelial cells of the islands of Langerhans, and also a slight hyperemia in two cases (16 and 17), while the other two cases (18 and 19) were in a normal condition.

Suprarenal Glands: All adrenals were in a normal condition.

E. ON THE PRODUCTION OF THE HYALINE AND "AMYLOID-LIKE" DEGENERATION IN RABBITS BY THE INJECTION OF PANCREATIN, TRYPSIN AND AMYLOPSIN

Since Frisch,[13] in 1877, announced the experimental production of an amyloid-like substance in the cornea of rabbits injected in the cornea with fresh blood from a case of anthrax, we have a majority of investigations on the production of experimental amyloidosis with such materials as turpentine (Czerny,[14] Lubarsch,[15] Nowak[16]),

13. Frisch: Sitzungsb. d. k. Akad. d. Wissensch. Math. Naturw. Cl., Abt. 3, **76**: 1877.
14. Czerny: Arch. f. exper. Path. u. Pharmakol. **31**: 1893.
15. Lubarsch: Virchows Arch. f. path. Anat. **150**: 1897.
16. Nowak: Virchows Arch. f. path. Anat. **153**: 1898.

B. pyocyaneus (Bouchard-Charrian,[17] Krakow[18]), *B. tuberculosis* (Bouchard-Charrian[17]), *Staphylococcus aureus* (Krakow,[18] Davidsohn,[19] Maximow,[20] Dantschakow,[21] gonococcus (Davidsohn[19]), fresh and sterile pus (Nowak[16]), *B. pestis* (Schoukewitsch[22]), *B. coli* (Bailey[23]), diphtheria toxin (Zenoni[24]), Pease-Pearce,[25] Lewis,[26] typhoid toxin (Lewis[26]), tuberculin (Nowak[16]), and lab ferment, papayotin and pancreatin (Schepilewsky[9]).

I could not find any report on the production of amyloidosis by pancreatin except Schepilewsky's experiment. He succeeded in producing an amyloidosis in the spleen in one experiment in six rabbits injected with pancreatin. He concluded that a hyaline degeneration caused more than an amyloid degeneration. I also produced a hyaline and an amyloid-like degeneration in rabbits injected with pancreatin, trypsin, and amylopsin as shown in the foregoing statement. An amyloid-like degeneration was produced in the livers of five rabbits (3, 4, 8, 24 and 25), in the kidneys of six rabbits (1, 3, 5, 6, 11 and 25), in the myocardium of five cases (3, 4, 8, 9, 10), and in the bone marrow in all the cases, especially marked in seven cases (1, 2, 4, 5, 6, 24 and 25), which stained a dark reddish-violet color with Pianese's solution, a slight reddish violet with gentian-violet, an intense red with eosin and a yellowish brown with Van Gieson's picro-fuchsin. Both the hyalin-like and amyloid-like degeneration in the livers of three rabbits (5, 9 and 10), and in the kidneys of two cases (2 and 4) stained a brick red and a slight dark reddish violet, or a dark red, with Pianese's solution, a dark green with gentian-violet and almost the same with eosin and van Gieson's picro-fuchsin as in the amyloid-like degeneration. A hyalin-like degeneration in the livers of three cases (1, 2 and 6), in the kidneys of three cases (8, 9 and 10), and in the myocardium of five cases (2, 5, 6, 11 and 25) stained a brick-red color with Pianese's solution, but a dark green with gentian-violet and almost the same with eosin and Van Gieson's picro-fuchsin as in the amyloid-like degeneration. An intermediary condition between the hyalin-like and amyloid-like degeneration in the spleen of ten cases (1, 2, 3, 4, 5, 6, 9, 10, 24 and 25) stained a dark red with Pianese's

17. Bouchard-Charrian: Compt. rend. Soc. de biol. **5**: 1888.
18. Krakow: Centralbl. f. allg. Path. u. path. Anat. **6**: 1895; Arch. de méd. exper. **8**: 1896; Arch. f. exper. Path. u. Pharmakol. **40**: 1898.
19. Davidsohn: Virchows Arch. f. path. Anat. **150**: 1897; Verhandl. d. Deutsch. path. Gesellsch. **7**: 1904; Ergebn. d. allg. Path. u. path. Anat. **12**: 1908; Virchows Arch. f. path. Anat. **192**: 1908.
20. Maximow: Virchows Arch. f. path. Anat. **153**: 1898.
21. Dantschakow: Virchows Arch. f. path. Anat. **187**: 1906.
22. Schoukewitsch: Arch. d. sc. biol. **12**: 1907.
23. Bailey: J. Exper. M. **23**: 1916, No. 6.
24. Zenoni: Centralbl. f. allg. Path. u. path. Anat. **13**: 1902.
25. Pease-Pearce: J. Infect. Dis. **3**: 1906.
26. Lewis: J. M. Res. **15**: 1906.

solution, a dark green with gentian-violet. Eosin and Van Gieson's picro-fuchsin stained them as in the other degenerative cases, as also a hyaline degeneration of the media of the medium-sized portal veins of the liver in one case (25), and also a larger hyaline mass in the glomeruli of the kidney of one case (6) of twelve rabbits injected with pancreatin intravenously. There was no such change present in the liver in one case (11), in the kidneys of one case (24), in the spleen of two cases (8 and 11) and in the myocardium of two cases (1 and 24), or in the lungs, adrenals, and pancreas of all the cases.

By the injection of trypsin there was also produced an amyloid-like degeneration of the liver and bone marrow of all the cases, an intermediary degeneration between the hyalin-like and amyloid-like degeneration in the spleen of two cases (21 and 22), and a hyalin-like degeneration in the spleen of one case (20), and in the myocardium of all the cases (three rabbits), while no such degeneration was found in the lungs, adrenals or the pancreas of all the cases; by the injection of amylopsin, amyloid-like degeneration was produced in the kidneys of three rabbits (16, 17 and 19), in the bone marrow of all the cases and in the media of the medium pulmonary arteries in one case (19). A degeneration intermediary between the hyalin-like and amyloid-like degeneration was produced in the livers of all the animals; a hyaline degeneration in the myocardium of one case (17) and in the islands of Langerhans of the pancreas in one case (16), while no such degeneration occurred in spleen and adrenals of any of the cases, but did occur in the kidneys of one case (18), in the myocardium of three cases (16, 18 and 19), in the lungs of three cases (16, 17 and 18), and in the pancreas of three cases (17, 18 and 19).

Summarizing the foregoing results, the intravenous injection of pancreatin, trypsin and amylopsin could produce a hyalin-like and an amyloid-like degeneration in the bone marrow, liver, kidneys, spleen and myocardium of most of the cases and also in the blood vessels and pancreas in a few cases.

The reason I call the substance stained a slight reddish violet with Pianese's solution and gentian-violet an "amyloid-like" substance is that if the amyloid-like degeneration is a hyaline degeneration, the degenerated cells or tissues should be stained a brick red, because Pianese's solution gives a characteristic color to each substance; that is, a brick red to hyalin, a bright green to colloid and a dark reddish violet to a substance resembling amyloid. If this change is an amyloid degeneration, those cells and tissues should be stained a red color with gentian-violet, whereas they have been stained a dark reddish violet or a slight reddish violet with Pianese's solution. Therefore it should be described as an intermediate degeneration between hyaline and amyloid degeneration, or rather an "amyloid-like" degeneration.

At present, however, we have no sufficient knowledge of the chemistry of amyloid and hyalin or colloid, although von Recklinghausen regards them as representing but different stages in the evolution of the same body.

Their differentiation by staining reactions at present, therefore, is not fully characteristic or always as satisfactory as Davidsohn,[19] Krakow[18] and Maximow[20] have believed, for they are closely allied bodies; however, Pianese[27] has recently differentiated these materials with his staining solution, which is the only method available at present.

Thus the amyloid-like degeneration produced in my experiments probably is an early stage of amyloid degeneration, and therefore the hyalin-like substance should be a precursor of amyloid, as Lubarsch[28] has suggested.

Considering the facts, amyloid probably passes through a hyaline stage in its formation, and these hyalin-like and amyloid-like substances seem to me likewise to be products of cell degeneration.

F. ON THE PRODUCTION OF ARTERIOSCLEROSIS IN RABBITS BY THE INJECTION OF PANCREATIN, TRYPSIN AND AMYLOPSIN

Up to the present we have had many investigations and observations on the production of experimental arteriosclerosis by the injection of extracts of vegetable and animal substances, bacteria and bacterial toxins, etc., or feeding with animal substances and lipoids, and also by various mechanisms. I could, however, find no reports on the production of arteriosclerosis by ferments. I produced arteriosclerosis in the rabbits by the repeated injection of pancreatin, trypsin and amylopsin. In the foregoing experiments there were presented thickening of the intima together with an increase of the media of the smaller or medium sized pulmonary arteries in four cases (5, 11, 24 and 25) in twelve rabbits injected with pancreatin; in two cases (20 and 21) of three rabbits injected with trypsin, in the smaller or medium-sized pulmonary, hepatic and splenic arteries in one case (16), of the renal and pulmonary arteries in one case (17), and also of the pulmonary arteries in one case (18) of four rabbits injected with amylopsin. These rabbits were repeatedly injected into the ear veins with the antigens. Unfortunately, I did not examine at necropsy the larger arteries of these rabbits. In these cases, no fatty degeneration was recognized in the lesion of the intima.

Jores,[29] in 1903, asserted concerning the development of arteriosclerosis that fatty degeneration of the thickened intima is an impor-

27. Pianese: Mallory and Wright's Pathological Technique, Ed. 6, 1915, p. 412.
28. Lubarsch: Centralbl. f. allg. Path. u. path. Anat. **21**: 1910, No. 3.
29. Jores: Wesen und Entwicklung der Arteriosklerose, Wiesbaden, 1903.

tant factor for the determination of arteriosclerosis, but Torhorst,[30] in 1904, observed that arteriosclerosis of the pulmonary arteries showed no fatty degeneration in the intima. According to Tawara's[12] assertion on this subject (which was further studied by him in 1913), a thickened intima which shows no fatty degeneration is also arteriosclerosis.

Tawara's assertion, therefore, suggested to me that the thickening of the intima and some of the media, without fatty degeneration, of the smaller or medium pulmonary, hepatic, renal and splenic arteries in my experiments should certainly be considered as arteriosclerosis; the thickening of the intima would be the stage of arteriosclerosis corresponding to Jore's[29] hyperplastic intima thickening, or to Kon's[31] second type, even if there were no fatty degeneration; however, there would appear to be a fatty degeneration following degenerative processes in the intima.

The essential conditions for the production of arteriosclerosis are not determined at present, and I should not dare to assume that they are determined by my experiments; but I venture the opinion that the arteriosclerosis produced in the experiments recorded here was due to pancreatin, trypsin and amylopsin acting on the intima of the vessel walls as a poison, with resulting hypertension, subacute or chronic progressive changes in the interstitial connective tissues and subsequent retrogressive changes in the parenchymatous cells, especially of the liver and kidneys. On the production of the arteriosclerosis in rabbits, however, one would have a question, because normal rabbits have sometimes a spontaneous arterisclerosis, a condition which was investigated by Bennecke,[32] who found arteriosclerosis in twelve out of 400 apparently normal rabbits. In 1908 Kon[31] found it in one normal rabbit during his researches on experimental arteriosclerosis. Other workers have also described the condition.

I cannot, however, believe that the arteriosclerosis produced in my experiment is a spontaneous arteriosclerosis, because it was produced in about 33 per cent. of the animals (four out of twelve rabbits) with pancreatin, in about 66 per cent. (two of three rabbits) with trypsin, and in 75 per cent. (three out of four rabbits) with amylopsin. I could find no spontaneous arteriosclerosis in fifty-three rabbits killed within three days after one injection of pancreatin, nor in nine normal rabbits employed in the foregoing experiments. Loeb[33] also could not find spontaneous arteriosclerosis in 280 apparently normal rabbits;

30. Torhorst: Beitr. z. path. Anat. u. z. allg. Path. **36**: 1904.
31. Kon: Verhandl. d. Jap. path. Gesellsch. **3**: 1913.
32. Bennecke: Virchows Arch. f. path. Anat. **191**: 1908, Part 2.
33. Loeb, O.: From Bennecke's article in Virchows Arch. f. path. Anat. **191**: 1908, Part 2.

therefore, I conclude that the arteriosclerosis found in my experiments is an artificial arteriosclerosis, but not spontaneous.

G. SUMMARY AND CONCLUSIONS

1. In rabbits, when introduced into the blood stream, both native and alcoholic pancreatin give rise to toxicity, such as trembling, uneasiness, difficulty in walking, dyspnea, and strong palpitation of the heart, and sometimes to shock a short time after the injection; however, the alcoholic extract is much less toxic than the native, but the toxicity of the latter decreases after the promotion of immune processes in the body.

2. Sometimes an acute inflammation, followed by abscess or gangrene is produced by subcutaneous injections.

3. A dose 0.05 gm. of native pancreatin to 1 kg. of body weight seems to be the minimal lethal dose in rabbits injected intravenously.

4. Both native and alcoholic pancreatin produce hyperemia, hemorrhage and thrombosis throughout the bodies of rabbits injected once, and sometimes make the blood incoagulable.

5. Fat infiltration was found in the livers of some rabbits killed within three days after one injection.

6. Some rabbits injected with pancreatin developed a crippled condition in the legs during immunization, which was due to amyloid degeneration of the bone marrow.

7. Some enlargement of the spleen and adrenals and amyloid degeneration of the bone marrow in most cases, and also jaundice in one case immunized with pancreatin, were found at necropsy.

8. Immunized rabbits in general showed some hyperplasia in the interstitial tissues of the liver, kidneys, spleen, lung, myocardium, bone marrow and pancreas, especially a subacute interstitial and parenchymatous hepatitis and nephritis in most cases, some necrosis of the bone marrow in the majority of cases, and an increase of phagocytic and giant cells in the spleen in all the cases. The rabbits also showed amyloid degeneration in the bone marrow, an amyloid-like and a hyaline degeneration in the liver, kidneys, spleen and myocardium in the majority of cases, and also fatty degeneration in the liver, kidneys, spleen and myocardium in all the cases; an arteriosclerosis of the smaller or medium pulmonary arteries in four cases, and a hyaline degeneration of the portal vein in one case out of twelve.

9. Trypsin and amylopsin give rise to the same toxicity and pathologic changes as pancreatin.

10. Rabbits immunized with trypsin developed an amyloid-like degeneration in the liver, kidneys, spleen and bone marrow, but a hyaline degeneration in the myocardium, an arteriosclerosis in the

smaller or medium pulmonary arteries, a fatty degeneration in the liver, kidneys, spleen and myocardium in the majority of cases, some enlargement of spleen and kidneys in most cases and a jaundice in one case.

11. Rabbits immunized with amylopsin showed an amyloid degeneration in the bone marrow, an amyloid-like degeneration in the liver, in the kidneys of most cases, and in the media of the medium-sized pulmonary arteries in one case; a hyaline degeneration in the myocardium and pancreas, one case of each, and an arteriosclerosis of the smaller or medium pulmonary, hepatic, splenic and renal arteries in a few cases; also a fatty degeneration in the liver, spleen and myocardium of most of the other cases.

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